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<p>(21) International Application Number: PCT/US97/18145 (22) International Filing Date: 8 October 1997 (08.10.97) (30) Priority Data: 60/027,981 8 October 1996 (08.10.96) US (71) Applicant: UNIVERSITY OF WASHINGTON [US/US]; 1107 N.E. 45th Street, Seattle, WA 98105 (US). (72) Inventors: CASTILLO, Gerardo; 6500 24th Avenue N.W. #104, Seattle, WA 98117 (US). SNOW, Alan, D.; 3812 - 167th Place S.W., Lynnwood, WA 98037 (US). (74) Agent: DWYER, Patrick, M.; Dwyer Marquardt, PLLC, 1919 One Union Square, 600 University Street, Seattle, WA 98101 (US).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report.</p>
<p>(54) Title: THERAPEUTIC AND DIAGNOSTIC APPLICATIONS OF LAMININ AND LAMININ-DERIVED PROTEIN FRAGMENTS</p>		
<p>(57) Abstract</p> <p>The invention relates to the discovery, identification and use of laminin, laminin-derived protein fragments, and laminin-derived polypeptides, as well as related peptides and antibodies, for the therapeutic intervention and diagnosis of Alzheimer's disease and other amyloidoses. In addition, the discovery and identification of an Alzheimer's beta-amyloid protein specific binding within the globular domain repeats of the laminin A chain, had led to new diagnostic and therapeutic applications for Alzheimer's disease and other amyloidoses which are disclosed.</p>		

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Title: **THERAPEUTIC AND DIAGNOSTIC APPLICATIONS OF LAMININ
AND LAMININ-DERIVED PROTEIN FRAGMENTS**

TECHNICAL FIELD

5 The invention relates to the discovery, identification and use of laminin, laminin-derived protein fragments, and laminin-derived polypeptides, as well as related peptides and antibodies, for the therapeutic intervention and diagnosis of Alzheimer's disease and other amyloidoses. In addition, the discovery and identification of an Alzheimer's beta-amyloid protein (A β) specific binding region
10 within the globular domain repeats of the laminin A chain, has led to new diagnostic and therapeutic applications for Alzheimer's disease and other amyloidoses which are disclosed.

BACKGROUND OF THE INVENTION

 Alzheimer's disease is characterized by the accumulation of a 39-43 amino acid
15 peptide termed the beta-amyloid protein or A β , in a fibrillar form, existing as extracellular amyloid plaques and as amyloid within the walls of cerebral blood vessels. Fibrillar A β amyloid deposition in Alzheimer's disease is believed to be detrimental to the patient and eventually leads to toxicity and neuronal cell death, characteristic hallmarks of Alzheimer's disease. Accumulating evidence now
20 implicates amyloid as a major causative factor of Alzheimer's disease pathogenesis. Discovery and identification of new compounds, agents, proteins, polypeptides or

protein-derivatives as potential therapeutic agents to arrest Alzheimer's disease A β amyloid formation, deposition, accumulation and/or persistence is desperately sought.

It is known that A β is normally present in human blood and cerebrospinal fluid. However, it is not known why this potential fibrillar protein remains soluble
5 in circulating biological fluids. Can the agent(s) responsible for this extraordinary solubility of fibrillar A β be applied to diagnostic and therapeutic regimens against the fibrillar A β amyloid present in Alzheimer's brain?

DISCLOSURE OF THE INVENTION

Summary of the Invention

10 The present invention provides answers to these questions and relates to the novel and surprising discovery that laminin and specific laminin-derived protein fragments are indeed potent inhibitors of Alzheimer's disease amyloidosis, and therefore have potential use for the therapeutic intervention and diagnosis of the amyloidoses. In addition, we have identified a specific region within laminin which
15 interacts with the Alzheimer's disease beta-amyloid protein and contributes to the observed inhibitory and therapeutic effects. In addition, specific laminin-derived protein fragments which also interact with the A β of Alzheimer's disease have been discovered to be present in human serum and cerebrospinal fluid, and implicate diagnostic applications which are described.

20 Laminin is a specific basement membrane component that is involved in several fundamental biological processes, and may play important roles in the pathogenesis of a number of different human diseases. Using a solid phase binding immunoassay, the present invention determined that laminin binds the A β of Alzheimer's disease with a single binding constant of $K_d = 2.7 \times 10^{-9}$ M. In addition,
25 using a Thioflavin T fluorometry assay (which quantitatively determines the amount of fibrillar amyloid formed), the present invention has determined that laminin is

surprisingly an extremely potent inhibitor of A β fibril formation. In this latter study, 25 μ M of A β (residues 1-40) was incubated at 37°C for 1 week in the presence or absence of 100 nM laminin. Laminin was found to significantly ($p < 0.001$) inhibit A β (1-40) amyloid fibril formation by 2.9-fold at 1 hour, 4.6-fold at 1 day, 30.6-fold at 3
5 days and 27.1-fold at 1 week. Other basement membrane components including perlecan, fibronectin and type IV collagen were not effective inhibitors of A β (1-40) fibrillogenesis in comparison to laminin, demonstrating the specificity of the inhibitory effect exhibited by laminin. The inhibitory effects of laminin on A β fibrillogenesis was also found to occur in a dose-dependent manner. In addition,
10 laminin was found to cause dissolution of pre-formed Alzheimer's disease amyloid fibrils in a dose-dependent manner following 4 days of incubation. Laminin was digested with V8, trypsin or elastase to determine small protease-resistant fragments of laminin which still interacted with A β . A ~55 kilodalton (kDa) laminin fragment derived from V8 or elastase digested laminin was found to interact with biotinylated
15 A β (1-40). Amino acid sequencing of the ~55 kDa fragment identified an A β -binding domain within laminin situated within the globular repeats of the laminin A chain.

Intact laminin was found to be present in human serum but not human cerebrospinal fluid, whereas laminin protein fragments ranging from ~120 kDa to ~200 kDa were found to be present in both human serum and cerebrospinal fluid. Of all
20 the laminin protein fragments present in human biological fluids described above, a prominent ~130 kilodalton band was found in human serum and cerebrospinal fluid which primarily interacted with A β as determined by ligand blotting methodology. This ~130 kilodalton laminin fragment is known as the E8 fragment (i.e. generated following elastase digestion of laminin)(Yurchenco and Cheng, J. Biol. Chem.
25 268:17286-17299, 1993) and is also believed to consist of the globular domains of the laminin A chain. The interaction of specific laminin fragments such as the newly discovered ~130 kDa protein is believed to bind A β in biological fluids and keep it in

a soluble state. The present invention describes the use of laminin, laminin-derived protein fragments, and laminin-derived polypeptides for the therapeutic intervention and diagnosis of Alzheimer's disease and other amyloidoses. In addition, the discovery and identification of a specific Alzheimer's A β -binding region within the globular domain repeats of the laminin A chain, and the discovery of the presence of laminin fragments containing this region in human serum and cerebrospinal fluid, has led to new diagnostic and therapeutic applications for Alzheimer's disease and other amyloidoses.

Features of the Invention

A primary object of the present invention is to establish new therapeutic methods and diagnostic applications for the amyloid diseases. The amyloid diseases include, but are not limited to, the amyloid associated with Alzheimer's disease and Down's syndrome (wherein the specific amyloid is referred to as beta-amyloid protein or A β), the amyloid associated with chronic inflammation, various forms of malignancy and Familial Mediterranean Fever (wherein the specific amyloid is referred to as AA amyloid or inflammation-associated amyloidosis), the amyloid associated with multiple myeloma and other B-cell dyscrasias (wherein the specific amyloid is referred to as AL amyloid), the amyloid associated with type II diabetes (wherein the specific amyloid is referred to as amylin or islet amyloid), the amyloid associated with the prion diseases including Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, kuru and animal scrapie (wherein the specific amyloid is referred to as PrP amyloid), the amyloid associated with long-term hemodialysis and carpal tunnel syndrome (wherein the specific amyloid is referred to as beta₂-microglobulin amyloid), the amyloid associated with senile cardiac amyloid and Familial Amyloidotic Polyneuropathy (wherein the specific amyloid is referred to as transthyretin or prealbumin), and the amyloid associated with endocrine tumors

such as medullary carcinoma of the thyroid (wherein the specific amyloid is referred to as variants of procalcitonin).

A primary object of the present invention is to use laminin, laminin-derived protein fragments and/or laminin-derived polypeptides as potent inhibitors of amyloid
5 formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses. "Laminin fragments, laminin-derived fragments, laminin-derived protein fragments and/or laminin-derived polypeptides", may include, but are not limited to, laminin A (or A1) chain, laminin B1 chain, laminin B2 chain, laminin A2 chain (merosin), laminin G1 chain, the globular domain repeats within the laminin
10 A1 chain, SEQ ID NO: 1 (11 amino acid sequence within the mouse laminin A chain), SEQ ID NO: 2 (fourth globular repeat with the mouse laminin A chain), SEQ ID NO: 3 (fourth globular repeat within the human laminin A chain), SEQ ID NO: 4 (mouse laminin A chain), SEQ ID NO: 5 (human laminin A chain), SEQ ID NO: 6 (human laminin B1 chain), SEQ ID NO: 7 (mouse laminin B1 chain), SEQ ID NO: 8 (rat
15 laminin B2 chain), SEQ ID NO: 9 (human laminin B2 chain), SEQ ID NO: 10 (mouse laminin G1 chain), SEQ ID NO: 11 (human laminin G1 chain), and all fragments or combinations thereof.

Yet another object of the present invention is to use conformational dependent proteins, polypeptides, or fragments thereof for the treatment of Alzheimer's disease
20 and other amyloidoses. Such conformational dependent proteins include, but are not limited to, laminin, laminin-derived fragments including laminin A1 chain (SEQ ID NO 4; SEQ ID NO: 5), the globular repeat domains within the laminin A1 chain (SEQ ID NO: 2, SEQ ID NO:3), an 11- amino acid peptide sequence within the globular domain of the laminin A chain (SEQ ID NO:1), laminin B1 chain (SEQ ID NO:6, SEQ
25 ID NO: 7), laminin B2 chain (SEQ ID NO: 8, SEQ ID NO:9), laminin G1 chain (SEQ ID NO: 10, SEQ ID NO: 11) and/or portions thereof.

Yet another aspect of the present invention is to use peptidomimetic compounds modelled from laminin, laminin-derived protein fragments and/or laminin-derived polypeptides, including but not limited to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses.

Yet another object of the present invention is to mimic the 3-dimensional A β -binding site(s) on laminin, laminin-derived protein fragments and/or laminin-derived polypeptides and use these mimics as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses.

Yet a further aspect of the present invention is to use anti-idiotypic antibodies to laminin, laminin-derived protein fragments and/or laminin-derived polypeptides as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses.

Another aspect of the invention is to provide new and novel polyclonal and/or monoclonal peptide antibodies which can be utilized in a number of in vitro assays to specifically detect A β -binding laminin derived protein fragments and/or A β -binding laminin derived polypeptides in human tissues and/or biological fluids. Polyclonal or monoclonal antibodies that are made specifically against a peptide portion or fragment of laminin which interacts with A β can be utilized to detect and quantify amyloid disease specific laminin fragments in human tissues and/or biological fluids. These antibodies can be made by administering the peptides in antigenic form to a suitable host. Polyclonal or monoclonal antibodies may be prepared by standard techniques known to those skilled in the art.

Another object of the present invention is to use laminin, the A β -binding laminin fragments and/or laminin-derived polypeptides referred to above, for the detection and specific localization of laminin peptides important in the amyloid diseases in human tissues, cells, and/or cell culture using standard immunohistochemical techniques.

Yet another aspect of the present invention is to use antibodies recognizing laminin, any of the A β -binding laminin fragments, and/or laminin-derived polypeptides including, but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, for in vivo labelling; for example, with a radionucleotide, for radioimaging to be utilized for in vivo diagnosis, and/or for in vitro diagnosis.

Yet another aspect of the present invention is to make use of laminin, A β -binding laminin protein fragments and/or A β -binding laminin-derived polypeptides including, but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, as potential therapeutics to inhibit the deposition, formation, and accumulation of fibrillar amyloid in Alzheimer's disease and other amyloidoses (described above), and to enhance the clearance and/or removal of preformed amyloid deposits in brain (for Alzheimer's disease and Down's syndrome amyloidosis) and in systemic organs (for systemic amyloidoses).

Another object of the present invention is to use A β -binding laminin-derived polypeptides or fragments thereof, in conjunction with polyclonal and/or monoclonal antibodies generated against these peptide fragments, using in vitro assays to detect amyloid disease specific autoantibodies in human biological fluids. Specific assay systems can be utilized to not only detect the presence of autoantibodies against

A β -binding laminin-derived protein fragments or polypeptides thereof in biological fluids, but also to monitor the progression of disease by following elevation or diminution of laminin protein fragments and/or laminin-derived polypeptide autoantibody levels.

5 Another aspect of the invention is to utilize laminin, laminin-derived protein fragments and/or laminin-derived polypeptide antibodies and/or molecular biology probes for the detection of these laminin derivatives in human tissues in the amyloid diseases.

10 Yet another object of the present invention is to use the laminin-derived protein fragments of the present invention in each of the various therapeutic and diagnostic applications described above. The laminin-derived protein fragments include, but are not limited to, the laminin A1 chain, the globular repeats within the laminin A1 chain, the laminin B1 chain, the laminin B2 chain, the laminin G1 chain, the laminin A2 chain (also known as merosin), and all constituents or variations
15 thereof, including but not limited to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, including peptides which have at least 70% homology to the sequences disclosed herein. Specific laminin-derived protein fragments or peptides as described above may be
20 derived from any species including, but are not limited to, human, murine, bovine, porcine, and/or equine species.

 Another object of the invention is to provide polyclonal and/or monoclonal peptide antibodies which can be utilized in a number of in vitro assays to specifically detect laminin protein fragments in human tissues and/or biological fluids. Polyclonal
25 or monoclonal antibodies made specifically against a peptide portion or fragment of any of the laminin fragments described herein can be utilized to detect and quantify laminin-derived protein fragments in human tissues and/or biological fluids. A

preferred embodiment is a polyclonal antibody made to the ~130 kilodalton A β -binding laminin fragment present in human serum and cerebrospinal fluid. These antibodies can be made by isolating and administering the laminin-derived fragments and/or polypeptides in antigenic form to a suitable host. Polyclonal or monoclonal antibodies
5 may be prepared by standard techniques by one skilled in the art.

Yet another object of the present invention is to use laminin-derived fragment antibodies as described herein as a specific indicator for the presence and extent of laminin breakdown in brain by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

10 Yet another object of the present invention is to use laminin-derived fragment antibodies as described herein as a specific indicator for the presence, extent and/or progression of Alzheimer's disease and/or other brain amyloidoses by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

15 Yet another object of the present invention is to use laminin-derived fragment antibodies as described herein as a specific indicator for the presence and extent of laminin breakdown in systemic organs by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

20 Yet another object of the present invention is to use laminin-derived fragment antibodies as described herein as a specific indicator for the presence and extent of amyloidosis in type II diabetes by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

25 Yet another object of the present invention is to use laminin-derived fragment antibodies as described herein as a specific indicator for the presence and extent of amyloidosis in other systemic amyloidoses by monitoring biological fluids including, but not limited to, cerebrospinal fluid, blood, serum, urine, saliva, sputum, and stool.

Yet another object of the present invention is to make use of peptides or fragments of laminin as described herein, including but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and
5 fragments thereof, as potential blocking therapeutics for the interaction of laminin and laminin-derived fragments in a number of biological processes and diseases (such as in Alzheimer's disease and other amyloid diseases described herein).

Yet another object of the invention is to utilize specific laminin-derived fragment antibodies, as described herein, for the detection of these laminin fragments
10 in human tissues in the amyloid diseases.

Another object of the present invention is to use laminin, laminin-derived protein fragments, and laminin-derived polypeptides, as described herein, for the treatment of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses.

15 Another object of the present invention is to use pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, syrups, tea bags, aerosols (as a solid or in a liquid medium), suppositories, sterile injectable solutions, and sterile packaged powders, which contain laminin, laminin-derived protein fragments, and laminin-derived
20 polypeptides, including, but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, to treat patients with Alzheimer's disease and other amyloidoses.

Yet another object of the present invention is to use laminin, laminin-derived
25 protein fragments, and laminin-derived polypeptides, including, but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ

ID NO: 11, and fragments thereof, as potent agents which inhibit amyloid formation, amyloid deposition, amyloid accumulation, amyloid persistence, and/or cause a dissolution of pre-formed or pre-deposited amyloid fibrils in Alzheimer's disease, and other amyloidoses.

5 Yet another object of the present invention is to provide the use of laminin, laminin-derived protein fragments, and laminin-derived polypeptides, as described herein, for inhibition of amyloid formation, deposition, accumulation, and/or persistence, regardless of its clinical setting.

Yet another object of the present invention is to provide compositions and
10 methods involving administering to a subject a therapeutic dose of laminin, laminin-derived protein fragments, and laminin-derived polypeptides, which inhibit amyloid deposition, including but not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof.

15 Accordingly, the compositions and methods of the invention are useful for inhibiting amyloidosis in disorders in which amyloid deposition occurs. The proteins or polypeptides of the invention can be used therapeutically to treat amyloidosis or can be used prophylactically in a subject susceptible to amyloidosis. The methods of the invention are based, at least in part, in directly inhibiting amyloid fibril formation,
20 and/or causing dissolution of preformed amyloid fibrils.

Yet another object of the present invention is to provide pharmaceutical compositions for treating amyloidosis. The pharmaceutical compositions include a therapeutic compound of the invention in an amount effective to inhibit amyloid deposition and a pharmaceutically acceptable vehicle.

25 These and other features and advantages of the present invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention.

FIGURE 1 is a binding curve demonstrating the binding interaction of EHS
5 laminin to substrate bound A β (1-40). A single binding site with a $K_d = 2.7 \times 10^{-9}$ M is determined.

FIGURE 2 demonstrates the potent inhibition of A β amyloid fibril formation by laminin as determined by a Thioflavin T fluorometry assay over a 1 week experimental period.

10 FIGURE 3 compares the potent inhibition of A β amyloid fibril formation by laminin to other basement membrane components including fibronectin, type IV collagen and perlecan. Only laminin is found to have a potent inhibitory effect on A β fibrillogenesis as early as 1 hour after incubation.

FIGURE 4 is a graph of a 1 week Thioflavin T fluorometry assay utilized to
15 determine the potential dose-dependent effects of laminin on inhibition of A β amyloid fibril formation. Significant dose-dependent inhibition of A β (1-40) amyloid fibril formation is observed at 1 day, 3 days and 1 week of treatment with increasing concentrations of laminin.

FIGURE 5 is a graph of a Thioflavin T fluorometry assay utilized to determine
20 the potential dose-dependent effects of laminin on dissolution of pre-formed A β (1-40) amyloid fibrils within a 4 day incubation period. Laminin causes dissolution of pre-formed A β amyloid fibrils in a dose-dependent manner.

FIGURE 6 is a graph of a 1 week Thioflavin T fluorometry assay utilized to determine the effects of laminin on islet amyloid polypeptide (amylin) fibrillogenesis,
25 and determine whether laminin causes a dose-dependent inhibition of amylin fibril

formation. Laminin does not significantly inhibit amylin fibrillogenesis suggesting its specificity for Alzheimer's disease amyloidosis.

FIGURE 7 is a black and white photograph of laminin digested with V8 protease, separated by SDS-PAGE and following interaction with biotinylated A β (1-40). The smallest fragment of V8-resistant laminin that interacts with A β is a ~55 kilodalton fragment.

FIGURE 8 is a black and white photograph of laminin digested with trypsin, separated by SDS-PAGE and following interaction with biotinylated A β (1-40). The smallest fragment of trypsin-resistant laminin that interacts with A β is a ~30 kilodalton fragment.

FIGURE 9 is a black and white photograph of laminin digested with elastase, separated by SDS-PAGE and following interaction with biotinylated A β (1-40). A ~55 kilodalton laminin fragment (arrow) that binds biotinylated A β was identified and sequenced. Note also the presence of a ~130 kDa fragment (arrowheads) that binds A β following 1.5 hours of elastase digestion (lane 2). Panel A is a ligand blot using biotinylated A β as a probe, whereas panel B is Coomassie blue staining of the same blot in Panel A to locate the specific band(s) for sequencing.

FIGURE 10 shows the complete amino acid sequence of the mouse laminin A chain. Sequencing of the ~55 kilodalton A β -binding band shown in Figure 9 leads to the identification of an 11 amino acid segment (underline and arrowhead) within the laminin A chain. This A β binding region of laminin is situated within the globular domain repeats of the laminin A[^] chain.

FIGURE 11 shows schematic diagrams of laminin and the newly discovered "A β binding region" of laminin (shown in left panel; between the two arrowheads) which is situated within the last three globular domains of the laminin A chain.

FIGURE 12 is a black and white photograph of a Western blot demonstrating the presence of laminin (arrowheads) and/or laminin-derived protein fragments (bands

between the two arrows) in human serum (lanes 1-7; left side) and human cerebrospinal fluid (lanes 1-7; right side) obtained from Alzheimer's disease, type II diabetes and normal aged patients. A ~110-130 kilodalton range of laminin positive protein fragments (between the two arrows) is present in both human serum and cerebrospinal fluid, whereas intact laminin (arrowheads) is only present in serum but not in cerebrospinal fluid.

FIGURE 13 is a black and white photograph demonstrating that intact laminin (arrow) and a prominent ~130 kilodalton band (arrowhead) present in human Alzheimer's disease, type II diabetes and normal aged patient serum, bind A β . The A β -binding laminin and specific A β -binding laminin fragments in human serum were identified following separation by SDS-PAGE and interaction with nanomolar concentrations of biotinylated A β (1-40).

FIGURE 14 is a black and white photograph demonstrating the presence of a prominent ~130 kilodalton band (arrow) in human Alzheimer's disease and normal aged patient cerebrospinal fluid, identified following separation by SDS-PAGE and following interaction with nanomolar concentrations of biotinylated A β (1-40). This same ~130 kilodalton A β -binding protein is also present in human serum (Figure 13).

BEST MODE OF CARRYING OUT THE INVENTION

The following sections are provided by way of additional background to better appreciate the invention.

Alzheimer's Disease

Alzheimer's disease is the most common cause of dementia in middle and late life, and is manifested by progressive impairment of memory, language, visuospatial perceptions and behavior (A Guide to the Understanding of Alzheimer's Disease and Related Disorders, edited by Jorm, New York University Press, New York 1987). A diagnosis of probable Alzheimer's disease can be made on clinical criteria (usually by

the exclusion of other diseases, memory tests etc), but a definite diagnosis requires the histological examination of specific abnormalities in the brain tissue usually obtained at autopsy.

In Alzheimer's disease, the parts of the brain essential for cognitive processes such as memory, attention, language, and reasoning degenerate, robbing victims of much that makes us human, including independence. In some inherited forms of Alzheimer's disease, onset is in middle age, but more commonly, symptoms appear from the mid-60's onward. Alzheimer's disease is characterized by the deposition and accumulation of a 39-43 amino acid peptide termed the beta-amyloid protein, A β or β /A4 (Glenner and Wong, Biochem. Biophys. Res. Comm. 120:885-890, 1984; Masters et al, Proc. Natl. Acad. Sci. USA 82:4245-4249, 1985; Husby et al, Bull. WHO 71:105-108, 1993). A β is derived from larger precursor proteins termed beta-amyloid precursor proteins (or β PPs) of which there are several alternatively spliced variants. The most abundant forms of the β PPs include proteins consisting of 695, 751 and 770 amino acids (Tanzi et al, Nature 331:528-530, 1988; Kitaguchi et al, Nature 331:530-532, 1988; Ponte, et al, Nature 331:525-528, 1988). The small A β peptide is a major component which makes up the amyloid deposits of neuritic "plaques" and in the walls of blood vessels (known as cerebrovascular amyloid deposits) in the brains of patients with Alzheimer's disease. In addition, Alzheimer's disease is characterized by the presence of numerous neurofibrillary "tangles", consisting of paired helical filaments which abnormally accumulate in the neuronal cytoplasm (Grundke-Iqbal et al, Proc. Natl. Acad. Sci. USA 83:4913-4917, 1986; Kosik et al, Proc. Natl. Acad. Sci. USA 83:4044-4048, 1986; Lee et al, Science 251:675-678, 1991). The pathological hallmarks of Alzheimer's disease is therefore the presence of "plaques" and "tangles", with amyloid being deposited in the central core of plaques and within the blood vessel walls. It is important to note that a so-called "normal aged brain" has some amyloid plaques and neurofibrillary tangles present. However, in comparison, an

Alzheimer's disease brain shows an over abundance of plaques and tangles. Therefore, differentiation of an Alzheimer's disease brain from a normal brain from a diagnostic point of view is primarily based on quantitative assessment of "plaques" and "tangles".

5 In an Alzheimer's disease brain, there are usually thousands of neuritic plaques. The neuritic plaques are made up of extracellular deposits consisting of an amyloid core usually surrounded by enlarged axons and synaptic terminals, known as neurites, and abnormal dendritic processes, as well as variable numbers of infiltrating microglia and surrounding astrocytes. The neurofibrillary tangles present
10 in the Alzheimer's disease brain mainly consist of tau protein, which is a microtubule-associated protein (Grundke-Iqbal et al, Proc. Natl. Acad. Sci. USA 83:4913-4917, 1986; Kosik et al, Proc. Natl. Acad. Sci. USA 83:4044-4048, 1986; Lee et al, Science 251:675-678, 1991). At the ultrastructural level, the tangle consists of paired helical filaments twisting like a ribbon, with a specific crossing over periodicity
15 of 80 nanometers. In many instances within a neurofibrillary tangle, there are both paired helical filaments and straight filaments. In addition, the nerve cells will many times die, leaving the filaments behind. These tangles are known as "ghost tangles" since they are the filamentous remnants of the dead neuron.

The other major type of lesion found in the brain of an Alzheimer's disease
20 patient is the accumulation of amyloid in the walls of blood vessels, both within the brain parenchyma and in the walls of the larger meningeal vessels which lie outside the brain. The amyloid deposits localized to the walls of blood vessels are referred to as cerebrovascular amyloid or congophilic angiopathy (Mandybur, J. Neuropath. Exp. Neurol. 45:79-90, 1986; Pardridge et al, J. Neurochem. 49:1394-1401, 1987).

25 In addition, Alzheimer's disease patients demonstrate neuronal loss and synaptic loss. Furthermore, these patients also exhibit loss of neurotransmitters such as acetylcholine. Tacrine, the first FDA approved drug for Alzheimer's disease is a

cholinesterase inhibitor (Cutler and Sramek, New Engl. J. Med. 328:808-810, 1993). However, this drug has showed limited success, if any, in the cognitive improvement in Alzheimer's disease patients and initially had major side effects such as liver toxicity.

5 For many years there has been an ongoing scientific debate as to the importance of "amyloid" in Alzheimer's disease and whether the "plaques" and "tangles" characteristic of this disease, were a cause or merely the consequences of the disease. Recent studies during the last few years have now implicated that amyloid is indeed a causative factor for Alzheimer's disease and not merely an
10 innocent bystander. The Alzheimer's disease A β protein in cell culture has been shown to cause degeneration of nerve cells within short periods of time (Pike et al, Br. Res. 563:311-314, 1991; J. Neurochem. 64:253-265, 1994). Studies suggest that it is the fibrillar structure, a characteristic of all amyloids, that is responsible for the neurotoxic effects. The A β has also been found to be neurotoxic in slice cultures of
15 hippocampus (the major memory region affected in Alzheimer's)(Harrigan et al, Neurobiol. Aging 16:779-789, 1995) and induces nerve cell death in transgenic mice (Games et al, Nature 373:523-527, 1995; Hsiao et al, Neuron 15:1203-1218, 1995). In addition, injection of the Alzheimer's A β into rat brain causes memory impairment and neuronal dysfunction (Flood et al, Proc. Natl. Acad. Sci. U.S.A. 88:3363-3366,
20 1991; Br. Res. 663:271-276, 1994), two additional hallmarks of Alzheimer's disease. Probably, the most convincing evidence that amyloid (ie. beta-amyloid protein) is directly involved in the pathogenesis of Alzheimer's disease comes from genetic studies. It has been discovered that the production of A β can result from mutations in the gene encoding, its precursor, known as the beta-amyloid precursor protein (Van
25 Broeckhoven et al, Science 248:1120-1122, 1990; Europ. Neurol. 35:8-19, 1995; Murrell et al, Science 254:97-99, 1991; Haass et al, Nature Med. 1:1291-1296, 1995). This precursor protein when normally processed usually only produces very little of

the toxic A β . The identification of mutations in the amyloid precursor protein gene which causes familial, early onset Alzheimer's disease is the strongest argument that amyloid is central to the pathogenetic process underlying this disease. Four reported disease-causing mutations have now been discovered which demonstrate the importance of the beta-amyloid protein in causing familial Alzheimer's disease (reviewed in Hardy, Nature Genet. 1:233-234, 1992). All of these studies suggest that providing a drug to reduce, eliminate or prevent fibrillar A β formation, deposition, accumulation and/or persistence in the brains of human patients should be considered an effective therapeutic.

10 Other Amyloid Diseases

The "amyloid diseases" consist of a group of clinically and generally unrelated human diseases which all demonstrate a marked accumulation in tissues of an insoluble extracellular substance known as "amyloid", and usually in an amount sufficient to impair normal organ function. Rokitansky in 1842 (Rokitansky, "Handbuch der pathologischen Anatomie", Vol. 3, Braumuller and Seidel, Vienna) was the first to observe waxy and amorphous looking tissue deposits in a number of tissues from different patients. However, it wasn't until 1854 when Virchow (Virchow, Arch. Path. Anat. 8:416, 1854) termed these deposits as "amyloid" meaning "starch-like" since they gave a positive staining with the sulfuric acid-iodine reaction, which was used in the 1850's for demonstrating cellulose. Although cellulose is not a constituent of amyloid, nonetheless, the staining that Virchow observed was probably due to the present of proteoglycans (PGs) which appear to be associated with all types of amyloid deposits. The name amyloid has remained despite the fact that Friederich and Kekule in 1859 discovered the protein nature of amyloid (Friedrich and Kekule, Arch. Path. Anat. Physiol. 16:50, 1859). For many years, based on the fact that all amyloids have the same staining and structural properties, lead to the postulate that a single pathogenetic mechanism was involved in amyloid deposition,

and that amyloid deposits were thought to be composed of a single set of constituents. Current research has clearly shown that amyloid is not a uniform deposit and that amyloids may consist of different proteins which are totally unrelated (Glenner, N. England J. Med. 302:1283-1292, 1980).

5 Although the nature of the amyloid itself has been found to consist of completely different and unrelated proteins, all amyloids appear similar when viewed under the microscope due to amyloid's underlying protein able to adapt into a fibrillar structure. All amyloids regardless of the nature of the underlying protein 1) stain characteristically with the Congo red dye and display a classic red/green birefringence
10 when viewed under polarized light (Puchtler et al, J. Histochem. Cytochem. 10:355-364, 1962), 2) ultrastructurally consists of fibrils with a diameter of 7-10 nanometers and of indefinite length, 3) adopt a predominant beta-pleated sheet secondary structure. Thus, amyloid fibrils viewed under an electron microscope (30,000 times magnification) from the post-mortem brain of an Alzheimer's disease
15 patient would look nearly identical to the appearance of amyloid present in a biopsied kidney from a rheumatoid arthritic patient. Both these amyloids would demonstrate a similar fibril diameter of 7-10 nanometers.

 In the mid to late 1970's amyloid was clinically classified into 4 groups, primary amyloid, secondary amyloid, familial amyloid and isolated amyloid. Primary
20 amyloid, is amyloid appearing de novo, without any preceding disorder. In 25-40% of these cases, primary amyloid was the antecedent of plasma cell dysfunction such as the development of multiple myeloma or other B-cell type malignancies. Here the amyloid appears before rather than after the overt malignancy. Secondary amyloid, appeared as a complication of a previously existing disorder. 10-15% of patients with
25 multiple myeloma eventually develop amyloid (Hanada et al, J. Histochem. Cytochem. 19:1-15, 1971). Patients with rheumatoid arthritis, osteoarthritis, ankylosing spondylitis can develop secondary amyloidosis as with patients with tuberculosis, lung

abscesses and osteomyelitis (Benson and Cohen, Arth. Rheum. 22:36-42, 1979; Kamei et al, Acta Path. Jpn. 32:123-133, 1982; McAdam et al, Lancet 2:572-575, 1975). Intravenous drug users who self-administer and who then develop chronic skin abscesses can also develop secondary amyloid (Novick, Mt. Sin. J. Med. 46:163-167, 5 1979). Secondary amyloid is also seen in patients with specific malignancies such as Hodgkin's disease and renal cell carcinoma (Husby et al, Cancer Res. 42:1600-1603, 1982). Although these were all initially classified as secondary amyloid, once the amyloid proteins were isolated and sequenced many of these turned out to contain different amyloid proteins.

10 The familial forms of amyloid also showed no uniformity in terms of the peptide responsible for the amyloid fibril deposited. Several geographic populations have now been identified with genetically inherited forms of amyloid. One group is found in Israel and this disorder is called Familial Mediterranean Fever and is characterized by amyloid deposition, along with recurrent inflammation and high 15 fever (Mataxas, Kidney 20:676-685, 1981). Another form of inherited amyloid is Familial Amyloidotic Polyneuropathy, and has been found in Swedish (Skinner and Cohen, Biochem. Biophys. Res. Comm. 99:1326-1332, 1981), Portuguese (Saraiva et al, J. Lab. Clin. Med. 102:590-603, 1983; J. Clin. Invest. 74:104-119, 1984) and Japanese (Tawara et al, J. Lab. Clin. Med. 98:811-822, 1981) nationalities. Amyloid 20 deposition in this disease occurs predominantly in the peripheral and autonomic nerves. Hereditary amyloid angiopathy of Icelandic origin is an autosomal dominant form of amyloid deposition primarily affecting the vessels in the brain, and has been identified in a group of families found in Western Iceland (Jennson et al, Clin. Genet. 36:368-377, 1989). These patients clinically have massive cerebral hemorrhages in 25 early life which usually causes death before the age of 40.

The primary, secondary and familial forms of amyloid described above tend to involve many organs of the body including heart, kidney, liver, spleen,

gastrointestinal tract, skin, pancreas, and adrenal glands. These amyloid diseases are also referred to as "systemic amyloids" since so many organs within the body demonstrate amyloid accumulation. For most of these amyloidoses, there is no apparent cure or effective treatment and the consequences of amyloid deposition can be detrimental to the patient. For example, amyloid deposition in kidney may lead to renal failure, whereas amyloid deposition in heart may lead to heart failure. For these patients, amyloid accumulation in systemic organs leads to eventual death generally within 3 to 5 years.

Isolated forms of amyloid, on the other hand, tend to involve a single organ system. Isolated amyloid deposits have been found in the lung, and heart (Wright et al, Lab. Invest. 30:767-773, 1974; Pitkanen et al, Am. J. Path. 117:391-399, 1984). Up to 90% of type II diabetic patients (non-insulin dependent form of diabetes) have isolated amyloid deposits in the pancreas restricted to the beta cells in the islets of Langerhans (Johnson et al, New Engl. J. Med. 321:513-518, 1989; Lab. Invest. 66:522-535, 1992). Isolated forms of amyloid have also been found in endocrine tumors which secrete polypeptide hormones such as in medullary carcinoma of the thyroid (Butler and Khan, Arch. Path. Lab. Med. 110:647-649, 1986; Berger et al, Virch. Arch. A Path. Anat. Hist. 412:543-551, 1988). A serious complication of long term hemodialysis is amyloid deposited in the medial nerve and clinically associated with carpal tunnel syndrome (Gejyo et al, Biochem. Biophys. Res. Comm. 129:701-706, 1985; Kidney Int. 30:385-390, 1986). By far, the most common type and clinically relevant type of organ-specific amyloid, and amyloid in general, is that found in the brains of patients with Alzheimer's disease (see U.S. Patent No. 4,666,829 and Glenner and Wong, Biochem. Biophys. Res. Comm. 120:885-890, 1984; Masters et al, Proc. Natl. Acad. Sci., USA 82:4245-4249, 1985). In this disorder, amyloid is predominantly restricted to the central nervous system. Similar deposition of amyloid in the brain occurs in Down's syndrome patients once they reach the age of 35 years

(Rumble et al, New England J. Med. 320:1446-1452, 1989; Mann et al, Neurobiol. Aging 10:397-399, 1989). Other types of central nervous system amyloid deposition include rare but highly infectious disorders known as the prion diseases which include Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, and kuru (Gajdusek et al, Science 197:943-960, 1977; Prusiner et al, Cell 38:127-134, 1984; Prusiner, Scientific American 251:50-59, 1984; Prusiner et al, Micr. Sc. 2:33-39, 1985; Tateishi et al, Ann. Neurol. 24:35-40, 1988).

It was misleading to group the various amyloidotic disorders strictly on the basis of their clinical features, since when the major proteins involved were isolated and sequenced, they turned out to be different. For example, amyloid seen in rheumatoid arthritis and osteoarthritis, now known as AA amyloid, was the same amyloid protein identified in patients with the familial form of amyloid known as Familial Mediterranean Fever. Not to confuse the issue, it was decided that the best classification of amyloid should be according to the major protein found, once it was isolated, sequenced and identified.

Thus, amyloid today is classified according to the specific amyloid protein deposited. The amyloid diseases include, but are not limited to, the amyloid associated with Alzheimer's disease, Down's syndrome and hereditary cerebral hemorrhage with amyloidosis of the Dutch type (wherein the specific amyloid is now known as the beta-amyloid protein or A β), the amyloid associated with chronic inflammation, various forms of malignancy and Familial Mediterranean Fever (AA amyloid or inflammation-associated amyloidosis), the amyloid associated with multiple myeloma and other B-cell abnormalities (AL amyloid), the amyloid associated with type II diabetes (amylin or islet amyloid), the amyloid associated with the prion diseases including Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, kuru and animal scrapie (PrP amyloid), the amyloid associated with long-term hemodialysis and carpal tunnel syndrome (beta₂-microglobulin amyloid), the amyloid associated

with senile cardiac amyloid and Familial Amyloidotic Polyneuropathy (prealbumin or transthyretin amyloid), and the amyloid associated with endocrine tumors such as medullary carcinoma of the thyroid (variants of procalcitonin).

Laminin and Its Structural Domains

5 Laminin is a large and complex 850 kDa glycoprotein which normally resides on the basement membrane and is produced by a variety of cells including embryonic, epithelial and tumor cells (Foidart et al, Lab. Invest. 42:336-342, 1980; Timpl et al, Methods Enzymol. 82:831-838, 1982). Laminin-1 (is derived from the Engelbreth-Holm-Swarm tumor) and is composed of three distinct polypeptide chains, 10 A, B1 and B2 (also referred to as α 1, β 1 and γ -1, respectively), joined in a multidomain structure possessing three short arms and one long arm (Burgeson et al, Matrix Biol. 14:209-211, 1994). Each of these arms is subdivided into globular and rodlike domains. Studies involving in vitro self-assembly and the analysis of cell-formed basement membranes have shown that laminin exists as a polymer, 15 forming part of a basement membrane network (Yurchenco et al, J. Biol. Chem. 260:7636-7644, 1985; Yurchenco et al, J. Cell Biol. 117:1119-1133, 1992; Yurchenco and Cheng, J. Biol. Chem. 268: 17286-17299, 1993). Laminin is believed to play important roles in a number of fundamental biological processes including promotion of neural crest migration (Newgreen and Thiery, Cell Tissue Res. 211:269-291, 1980; 20 Rovasio et al, J. Cell Biol. 96:462-473, 1983), promotion of neurite outgrowth (Lander et al, Proc. Natl. Acad. Sci. 82:2183-2187, 1985; Bronner-Fraser and Lallier, Cell Biol. 106:1321-1329, 1988), the formation of basement membranes (Kleinman et al, Biochem. 22:4969-4974, 1983), the adhesion of cells (Engvall et al, J. Cell Biol. 103: 2457-2465, 1986) and is inducible in adult brain astrocytes by injury (Liesi et al, 25 EMBO J. 3:683-686, 1984). Laminin interacts with other components including type IV collagen (Terranova et al, Cell 22:719-726, 1980; Rao et al, Biochem. Biophys. Res. Comm. 128:45-52, 1985; Charonis et al, J. Cell Biol. 100: 1848-1853, 1985; Laurie

et al, J. Mol. Biol. 189:205-216, 1986), heparan sulfate proteoglycans (Riopelle and Dow, Brain Res. 525:92-100, 1990; Battaglia et al, Eur. J. Biochem. 208:359-366, 1992) and heparin (Sakashita et al, FEBS Lett. 116:243-246, 1980; Del Rosso et al, Biochem. J. 199:699-704, 1981; Skubitz et al, J. Biol. Chem. 263:4861-4868, 1988).

5 Several of the functions of laminin have been found to be associated with the short arms. First, the short arms have been found to participate in laminin polymerization (Yurchenco et al, J. Cell Biol. 117:1119-1133, 1992; Yurchenco and Cheng, J. Biol. Chem. 268: 17286-17299, 1993). A recently proposed three-arm interaction hypothesis of laminin polymerization (Yurchenco and Cheng, J. Biol.
10 Chem. 268: 17286-17299, 1993) further holds that self-assembly is mediated through the end regions of each of the three short arms. A prediction of this model is that each short arm can independently and competitively inhibit laminin polymerization. However, it has not been possible to formally test this prediction using conventional biochemical techniques because of an inability to separate the alpha and gamma
15 chains. Second, several heparin binding sites have been thought to reside in the short arms (Yurchenco et al, J. Biol. Chem. 265:3981-3991, 1990; Skubitz et al, J. Cell Biol. 115:1137-1148, 1991), although the location of these sites have remained obscure. Third, the alpha1 β 1 integrin has been found to selectively interact with large short arm fragments containing all or most of the short arm domains (Hall et al, J. Cell
20 Biol. 110:2175-2184, 1990; Goodman et al, J. Cell Biol. 113:931-941, 1991).

Most functional activities of laminin appear to be dependent upon the conformational state of the glycoprotein. Specifically, self-assembly and its calcium dependence, nidogen (entactin) binding to laminin, alpha6 β 1 integrin recognition of the long arm, heparin binding to the proximal G domain (cryptic) and RGD-dependent
25 recognition of the short A chain of laminin (cryptic) have all been found to be conformationally dependent (Yurchenco et al, J. Biol. Chem. 260:7636-7644, 1985; Fox et al, EMBO J. 10:3137-3146, 1991; Sung et al, J. Cell Biol. 123:1255-1268,

1993). Two consequences of improperly folded laminin, loss of normal functional activity and the activation of previously cryptic activities, suggest that it is important to map and characterize biological activities using correctly folded laminin or conformational homologues to any particular laminin or laminin fragment.

5 Laminin may also be involved in the pathogenesis of a number of important diseases. For example, in diabetes significant decrease in the levels of laminin on the glomerular basement membranes indicates that a molecular imbalance occurs (Shimomura and Spiro, Diabetes 36:374-381, 1987). In experimental AA amyloidosis (ie. inflammation-associated amyloidosis), increased levels of laminin are observed
10 at the sites of AA amyloid deposition (Lyon et al, Lab. Invest. 64:785-790, 1991). However, the role(s) of laminin in systemic amyloidosis is not known. In Alzheimer's disease and Down's syndrome, laminin is believed to be present in the vicinity of A β -containing amyloid plaques (Perlmutter and Chui, Brain Res. Bull. 24:677-686, 1990; Murtomaki et al, J. Neurosc. Res. 32:261-273, 1992; Perlmutter et al, Micro.
15 Res. Tech. 28:204-215, 1994).

 Previous studies have indicated that the various isoforms of the beta-amyloid precursor proteins of Alzheimer's disease, bind both the basement membrane proteins perlecan (Narindrasorasak et al, J. Biol. Chem. 266:12878-12883, 1991) and laminin (Narindrasorasak et al, Lab. Invest. 67:643-652, 1992). With regards to laminin, it
20 was not previously known whether laminin interacts with A β , whether a particular domain of laminin (if any) participates in A β interactions, and whether laminin had any significant role(s) in A β amyloid fibrillogenesis.

 The present invention has discovered that laminin binds A β with relatively high affinity and surprisingly laminin is a potent inhibitor of A β amyloid formation,
25 and causes dissolution of pre-formed Alzheimer's disease amyloid fibrils. In addition, a 55-kilodalton elastase resistant fragment of laminin which also binds A β has been localized to the globular domain repeats within the A chain of laminin. This region

is believed to be responsible for many of the inhibitory effects that laminin has on Alzheimer's disease amyloidosis. These findings indicate that laminin, laminin-derived protein fragments and/or laminin-derived polypeptides, particularly those containing the disclosed A β -binding site within the globular domain repeats within the laminin A chain, may serve as novel inhibitors of A β amyloidosis in Alzheimer's disease and other amyloidoses. In addition, the discovery and identification of an Alzheimer's A β -binding region within the globular domain repeats of the laminin A chain, and the discovery of its presence in human serum and cerebrospinal fluid, as a ~130 kDa laminin-derived fragment, leads to novel diagnostic and therapeutic applications for Alzheimer's disease and other amyloidoses.

Examples

The following examples are provided to disclose in detail preferred embodiments of the binding interaction of laminin with A β , and the potent inhibitory effects of laminin and disclosed fragments on A β fibril formation. However, it should not be construed that the invention is limited to these specific examples.

Example 1

Binding of Laminin to the Beta-Amyloid Protein (A β) of Alzheimer's Disease

2 μ g of A β (1-40)(Bachem Inc., Torrance, CA USA; Lot #WM365) in 40 μ l of Tris-buffered saline (TBS)(pH 7.0) was allowed to bind overnight at 4°C to microtiter wells (Nunc plates, Maxisorb). The next day all of the microtiter wells were blocked by incubating with 300 μ l of Tris-buffered saline containing 100 mM Tris-HCl, 50 mM NaCl, 0.05% Tween-20, and 3 mM NaN₃ (pH 7.4)(TTBS) plus 2% bovine serum albumin (BSA). Various dilutions (ie. 1:10, 1:30, 1:90, 1:270, 1:810, 1:2430 and 1:7290) of Engelbreth-Holm-Swarm (EHS) mouse tumor laminin (1 mg/ml)(Sigma Chemical Co., St. Louis, MO, USA) in 250 μ l of TBS (pH 7.4) were placed in wells (in triplicate) either containing substrate bound A β (1-40) or blank, and allowed to bind

overnight at 4°C overnight. The next day, the wells were rinsed 3 times with TTBS, and then probed for 2 hours with 100 µl of rabbit anti-laminin antibody (Sigma Chemical Company, St. Louis, MO) diluted 1:10,000 in TTBS. After 3 rinses with TTBS, the wells were then incubated for 2 hours on a rotary shaker with 100 µl of
5 secondary probe consisting of biotinylated goat anti-rabbit (1:1000) and strepavidin-peroxidase (1:500 dilution of a 2 µg/ml solution) in TTBS containing 0.1% BSA. The wells were then rinsed 3 times with TTBS and 100 µl of a substrate solution (OPD-Sigma Fast from Sigma Chemical Co., St. Louis, MO) was added to each well and allowed to develop for 10 minutes or until significant color differences
10 were observed. The reaction was stopped with 50 µl of 4N H₂SO₄ and read on a Model 450 microplate reader (Biorad, Hercules, CA USA) at 490 nm. Data points representing a mean of triplicate determinations were plotted and the affinity constants (ie. K_d) were determined using Ultrafit (version 2.1, Biosoft, Cambridge, U.K.) as described below.

15 The binding data were analyzed assuming a thermodynamic equilibrium for the formation of the complex BL, from the laminin ligand in solution, L, and the uncomplexed Aβ adsorbed to the microtiter well, B, according to the equation:

$$K_d = [B] \times [L] / [BL]$$

We elected to determine K_d's by using an enzyme-linked immunoassay that gives a
20 color signal that is proportional to the amount of unmodified laminin bound to Aβ (Engel, J. and Schalch, W., Mol. Immunol. 17:675-680, 1980; Mann, K. et al, Eur. J. Biochem. 178:71-80, 1988; Fox, J.W. et al, EMBO J. 10:3137-3146, 1991; Battaglia, C. et al, Eur. J. Biochem. 208:359-366, 1992).

To account for potential non-specific binding, control wells without Aβ (in
25 triplicate) were included for each concentration of laminin used in each binding experiment. Optical densities of the control wells never exceeded 0.050 at all laminin concentrations employed for these experiments. The optical densities of the control

wells were subtracted from the optical densities of the A β -containing wells that received similar laminin concentrations. Non-specific absorbance obtained from A β containing wells that did not receive laminin were also subtracted from all data points. Thus, the equation in the form of:

$$5 \quad OD_{exp} = OD_o + (S \times [laminin]) + (OD_{max} \times [laminin]/([laminin] + K_d))$$

where (S x [laminin]) represents non-specific binding (control wells) and OD_o is the non-specific absorbance, becomes:

$$OD_{exp} = OD_{max} \times [laminin]/([laminin] + K_d)$$

Therefore, at 50 % saturation, OD_{exp} = 0.50 OD_{max} and K_d = [laminin]. Determination of [laminin] at 50% saturation was performed by non-linear least square program (Ultrafit from Biosoft, UK) using a one-site model.

As demonstrated in Figure 1, EHS laminin bound immobilized A β (1-40) with a single binding constant with an apparent dissociation constant of K_d = 2.7 X 10⁻⁹ M. Several repeated experiments utilizing this solid phase binding immunoassay indicated that laminin bound A β (1-40) repetitively with one apparent binding constant.

Example 2

Inhibition of Alzheimer's Disease A β Fibril Formation by Laminin

The effects of laminin on A β fibrillogenesis was also determined using the previously described method of Thioflavin T fluorometry (Naiki et al, Lab. Invest. 65:104-110, 1991; Levine III, Protein Sci. 2:404-410, 1993; Levine III, Int. J. Exp. Clin. Invest. 2:1-6, 1995; Naiki and Nakakuki, Lab. Invest. 74:374-383, 1996). In this assay, Thioflavin T binds specifically to fibrillar amyloid and this binding produces a fluorescence enhancement at 480 nm that is directly proportional to the amount of amyloid fibrils formed (Naiki et al, Lab. Invest. 65:104-110, 1991; Levine III, Protein Sci. 2:404-410, 1993; Levine III, Int. J. Exp. Clin. Invest. 2:1-6, 1995; Naiki and Nakakuki, Lab. Invest. 74:374-383, 1996). In a first study, the effects of

EHS laminin on A β (1-40) fibrillogenesis was assessed. For this study, 25 μ M of freshly solubilized A β (1-40)(Bachem Inc., Torrance, CA, USA; Lot # WM365) was incubated in microcentrifuge tubes at 37°C for 1 week (in triplicate), either alone, or in the presence of 100 nM EHS laminin (Sigma Chemical Company, St. Louis, MO, USA) in 100 mM Tris, 50 mM NaCl, pH 7.0 (TBS). 100 nM of laminin utilized for these studies represented a A β :laminin molar ratio of 250:1. 50 μ l aliquots were then taken from each tube for analysis at 1 hr, 1 day, 3 days, and 1 week. In a second set of studies, the effects of laminin on A β (1-40) fibril formation was directly compared to other basement membrane components including fibronectin, type IV collagen and perlecan. For these studies, 25 μ M of freshly solubilized A β (1-40) was incubated in microcentrifuge tubes for 1 week (in triplicate) either alone, or in the presence of 100 nM of EHS perlecan (isolated as previously described)(Castillo et al, J. Biochem. 120:433-444, 1996), fibronectin (Sigma Chemical Company, St. Louis, MO, USA) or type IV collagen (Sigma Chemical Company, St. Louis, MO, USA). 50 μ l aliquots were then taken for analysis at 1 hour, 1 day, 3 days and 1 week. In a third set of studies, 25 μ M of freshly solubilized A β (1-40) was incubated in microcentrifuge tubes for 1 week (in triplicate) either alone, or in the presence of increasing concentrations of laminin (i.e. 5 nM, 15 nM, 40 nM and 100 nM). 50 μ l aliquots were taken for analysis at 1 hour, 1 day, 3 days and 1 week.

For each determination described above, following each incubation period, A β peptides +/- laminin, perlecan, fibronectin or type IV collagen, were added to 1.2 ml of 100 μ M Thioflavin T (Sigma Chemical Co., St. Louis, MO) in 50 mM phosphate buffer (pH 6.0). Fluorescence emission at 480 nm was measured on a Turner instrument-model 450 fluorometer at an excitation wavelength of 450 nm. For each determination, the fluorometer was calibrated by zeroing in the presence of the Thioflavin T reagent alone, and by setting the 50 ng/ml riboflavin (Sigma Chemical Co., St. Louis, Mo) in the Thioflavin T reagent to 1800 fluorescence units. All

fluorescence determinations were based on these references and any background fluorescence given off by laminin, perlecan, type IV collagen, or fibronectin alone in the presence of the Thioflavin T reagent was always subtracted from all pertinent readings.

5 As shown in Figure 2, freshly suspended A β (1-40) alone, following a 1 hour incubation at 37°C, demonstrated an initial fluorescence of 41 fluorescence units. During the 1 week incubation period there was a gradual increase in the fluorescence of 25 μ M A β (1-40) alone, increasing 6.7-fold from 1 hour to 1 week, with a peak fluorescence of 379 fluorescence units observed at 1 week. This increase was
10 significantly inhibited when A β (1-40) was co-incubated with laminin, in comparison to A β alone. A β (1-40) co-incubated with laminin displayed fluorescence values that were 2.9-fold lower ($p < 0.001$) at 1 hour, 4.6-fold lower ($p < 0.0001$) at 1 day, 30.6-fold lower ($p < 0.0001$) at 3 days and 27.1-fold lower ($p < 0.0001$) at 1 week. This study indicated that laminin was a potent inhibitor of A β amyloid fibril formation, nearly
15 completely inhibiting amyloid fibril formation even after 1 week of incubation.

To determine whether the inhibitory effects of laminin was specific to this basement membrane component, an direct comparison was made to other known basement membrane components including perlecan, fibronectin, and type IV collagen. In these studies 25 μ M of A β (1-40) was incubated in the absence or
20 presence of either 100 nM of laminin, 100 nM of fibronectin, 100 nM of type IV collagen and 100 nM of perlecan (Figure 3). Freshly solubilized A β (1-40) when incubated at 37°C gradually increased in fluorescence levels from 1 hour to 1 week (by 10.8-fold)(Figure 3), as previously demonstrated (Figure 2). Perlecan was found to significantly accelerate A β (1-40) amyloid formation at 1 day and 3 days, whereas
25 fibronectin and type IV collagen only showed significant inhibition of A β (1-40) fibrillogenesis at 1 week. Laminin, on the other hand, was again found to be a very potent inhibitor of A β fibrillogenesis causing a 9-fold decrease at 1 and 3 days, and

a 21-fold decrease at 1 week. This study reconfirmed the potent inhibitory effects of laminin on A β fibrillogenesis, and demonstrated the specificity of this inhibition, since none of the other basement membrane components (including fibronectin, type IV collagen and perlecan) were very effective inhibitors.

5 To determine whether the inhibitory effects of laminin on A β fibrillogenesis occurred in a dose-dependent manner, different concentrations of laminin (i.e. 5nM, 15 nM, 40 nM and 100 nM) were tested. As shown in Figure 4, freshly solubilized A β (1-40) when incubated at 37°C gradually increased from 1 hour to 1 week, as previously demonstrated (Figures 2 and 3). 100 nM of laminin significantly inhibited
10 A β fibril formation at all time points studied, including 1 hour, 1 day, 3 days and 7 days. Laminin was also found to inhibit A β fibril formation in a dose-dependent manner which was significant ($p < 0.05$) by 3 days of incubation. At 3 days and 7 days, both 100 nM and 40 nM of laminin significantly inhibited A β fibril formation. This study reconfirmed that laminin was a potent inhibitor of A β fibril formation and that
15 this inhibition occurred in a dose-dependent manner.

Example 3

Laminin Causes Dose-Dependent Dissolution of Pre-Formed Alzheimer's Disease Amyloid Fibrils

20 The next study was implemented to determine whether laminin was capable of causing a dose-dependent dissolution of pre-formed Alzheimer's disease A β (1-40) amyloid fibrils. This type of activity would be important for any potential anti-Alzheimer's amyloid drug which can be used in patients who already have substantial amyloid deposition in brain. For example, Alzheimer's disease patients in mid-to late stage disease have abundant amyloid deposits in their brains as part
25 of both neuritic plaques and cerebrovascular amyloid deposits. A therapeutic agent capable of causing dissolution of pre-existing amyloid would be advantageous for use in these patients who are at latter stages of the disease process.

For this study, 1 mg of A β (1-40)(Bachem Inc., Torrance, CA, USA; Lot #WM365) was dissolved in 1.0 ml of double distilled water (1mg/ml solution) and then incubated at 37°C for 1 week. 25 μ M of fibrillized A β was then incubated at 37°C in the presence or absence of laminin (from EHS tumor; Sigma Chemical Company, St. Louis, MO, USA) at concentrations of 125 nM, 63 nM, 31 nM and 16 nM containing 150 mM Tris HCl, 10 mM NaCl, pH 7.0. Following a 4 day incubation, 50 μ l aliquots were added to 1.2ml of 100 μ M Thioflavin T (Sigma Chemical Co., St. Louis, MO) in 50mM NaPO₄ (pH 6.0) for fluorometry readings as described in example 2.

As shown in Figure 5, dissolution of pre-formed Alzheimer's disease A β amyloid fibrils by laminin occurred in a dose-dependent manner. A significant (p<0.001) 41% dissolution of pre-formed A β amyloid fibrils was observed with 125 nM of laminin, whereas 63 nM of laminin caused a significant (p<0.001) 39% dissolution. Furthermore, 31 nM and 16 nM of laminin still caused a significant (p<0.01) 28% and 25% dissolution of pre-formed A β amyloid fibrils. These data demonstrated that laminin causes dissolution of pre-formed Alzheimer's disease amyloid fibrils in a dose-dependent manner following a 4-day incubation.

Example 4

Laminin Does Not Significantly Inhibit Islet Amyloid Polypeptide (Amylin) Fibril Formation

In the next study, the specificity of the laminin inhibitory effects on Alzheimer's disease amyloid was determined by testing laminin's potential effects on another type of amyloid. Amyloid accumulation occurs in the islets of Langerhans in ~90% of patients with type II diabetes (Westermarck et al, Am. J. Path. 127:414-417, 1987). The major protein in islet amyloid is a 37 amino acid peptide, termed islet amyloid polypeptide or amylin which is known to be a normal secretory product of the beta-cells of the pancreas (Cooper et al, Proc. Natl. Acad. Sci., 84:8628-8632, 1987).

The dose-dependent effects of laminin on amylin fibrillogenesis was determined using the Thioflavin T fluorometry assay. 25 μ M of A β (1-40)(Bachem Inc., Torrance, CA, USA; Lot #WM365) was incubated in microcentrifuge tubes at 37°C for 1 week (in triplicate), either alone, or in the presence of 5 nM, 15 nM, 40 nM and 100 nM of laminin in 150 mM Tris HCl, 10 mM NaCl, pH 7.0 (TBS). 50 μ l aliquots were taken from each tube for analysis at 1 hr, 1 day, 3 days, and 1 week using Thioflavin T fluorometry as described in example 2.

As shown in Figure 6, freshly suspended amylin alone following a 1-hour incubation at 37°C reached a maximum fluorescence of 1800 fluorescence units, which did not significantly change during the 1 week experimental period. The initial high fluorescence of amylin was attributed to amylin's ability to spontaneously form amyloid fibrils within a very short incubation period. Laminin at 100 nM did not significantly inhibit amylin fibril formation at all time points within the 1 week experimental period (Figure 6). In addition, no significant inhibition of amylin fibrillogenesis by laminin at decreasing concentrations (i.e. 40 nM, 15 nM and 5 nM) was observed, even though a decrease (but not significant) in amylin fibril formation was observed with 40 nM of laminin at 1 day, 3 days and 1 week (Figure 6). This study demonstrated that the inhibitory effects of laminin did not occur with amylin fibril formation, and demonstrated the specificity of the observed laminin inhibitory effects on Alzheimer's disease amyloid.

Example 5

Identification of V8 and Trypsin-Resistant Laminin Fragments which Interact with the Beta-Amyloid Protein of Alzheimer's Disease

In the next set of studies, we determined whether small fragment(s) of laminin generated by V8 or trypsin digestion would bind to A β . This would enable one to determine the domain(s) of laminin which bind A β and likely play a role in inhibition

of A β fibril formation and causing dissolution of preformed Alzheimer's amyloid fibrils (as demonstrated in the invention).

For these experiments, A β (1-40) was biotinylated according to the manufacturer's protocol (Pierce, Rockford, Illinois). For the ligand studies, intact
5 EHS laminin was left undigested, or digested with V8 or trypsin (Sigma Chemical Company, St. Louis, MO, USA). More specifically, 2 μ g of trypsin or V8 protease in 2 μ l of 50 mM Tris-HCl buffer (pH 8.0) were added to 50 μ l of laminin (50 μ g)(in the same buffer) and incubated overnight at 37°C. The next day, 10 μ l of protease-digested laminin (or undigested laminin) was mixed with 10 μ l of 2X sodium
10 dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, and heated for 5 minutes in a boiling water bath. SDS-PAGE was performed according to the method of Laemmli (Laemmli, U.K. Nature 227:680-685, 1970), or according to the method of Schagger and Jagow (Schagger and Jagow, Anal. Biochem. 166:368-379, 1987) using a Mini-Protean II electrophoresis system (Biorad) with
15 precast 4-15% Tris-Glycine or 10-20% tricine polyacrylamide gels, respectively, and under non-reducing conditions. Electrophoresis occurred at 200V for 45 minutes along with pre-stained molecular weight standards.

After SDS-PAGE (10-20% tricine or 4-15% Tris-Glycine gels) was performed as described above, the separated laminin and its fragments (total protein of 10
20 μ g/lane) were transferred to polyvinylidene difluoride membrane (PVDF) using a Mini transblot electrophoresis transfer cell (Biorad, Hercules, CA, U.S.A.). Electrotransfer was performed at 100V for 2 hours. Following transfer, membranes were rinsed with methanol and dried. The fragment(s) of laminin involved in binding to A β were then detected by using biotinylated-A β (1-40), as described above. Blots were probed for
25 2 hours with 2 μ M biotinylated A β (1-40) in TTBS. The membranes were then rinsed three times (10 seconds each) with TTBS, probed for 30 minutes with streptavidin alkaline phosphatase conjugate (Vectastain), rinsed again (as described above), and

followed by the addition of an alkaline phosphatase substrate solution (Vectastain). Following color development, the reaction was stopped by flushing the membranes with double distilled water.

As shown in Figure 7, V8-digested laminin produced multiple protein
5 fragments which interacted with biotinylated A β (1-40). Using a 4-15% Tris-Glycine gel system (Figure 7, lane 1), V8-resistant laminin fragments which interacted with A β included fragments of ~400 kDa (which probably represented intact laminin which was left undigested), ~100-130 kDa, ~85 kDa, and a prominent fragment at ~55 kDa. Using a 10-20% tricine gel system (Figure 7, lane 2), V8-resistant laminin fragments
10 which interacted with A β included fragments of ~130 kDa, ~85 kDa, and a prominent fragment at ~55 kDa (Figure 7, lane 2, arrow). It is important to note that molecular size expressed in kilodaltons (kDa) are generally approximate. This study demonstrated that the smallest V8-resistant protein fragment of laminin which interacted with A β (1-40) was ~55 kDa.

As shown in Figure 8, trypsin-digested laminin produced multiple protein
15 fragments which interacted with biotinylated A β (1-40). Using a 4-15% Tris-Glycine gel system (Figure 8, lane 1), trypsin-resistant laminin fragments which interacted with A β included fragments of ~400 kDa (which probably represented intact laminin which was left undigested), ~150-200 kDa, ~97 kDa, ~65 kDa and a prominent fragment
20 at ~30 kDa. Using a 10-20% tricine gel system (Figure 8, lane 2), trypsin-resistant laminin fragments which interacted with A β included fragments of ~97 kDa, ~90 kDa, ~65 kDa and a prominent fragment at ~30 kDa (Figure 8, lane 2, arrow). This study demonstrated that the smallest trypsin-resistant fragment of laminin which interacted with A β (1-40) was ~30 kDa.

Example 6**Identification of Elastase-Resistant Laminin Fragments Which Interact with the Beta-Amyloid Protein of Alzheimer's Disease**

In the next set of studies, we determined whether small fragment(s) of laminin
5 generated by elastase digestion would bind to A β . In addition, we sequenced and
identified the region within elastase-resistant laminin which interacted with A β . For
these experiments, A β (1-40) was biotinylated according to the manufacturer's
protocol (Pierce, Rockford, Illinois). For the ligand studies, intact EHS laminin was
left undigested, or digested with elastase (Sigma Chemical Company, St. Louis, MO,
10 USA). For elastase digestion, 2 μ g of elastase in 8 μ l of 50 mM Tris-HCl buffer (pH
8.0) was added to 50 μ l of laminin (50 μ g)(in the same buffer) and incubated for 1.5
hours or 2.5 hours at 37°C. In addition, as a control, 2 μ g of elastase in 50 μ l of 50
mM Tris-HCl buffer (pH 8.0) was incubated for 2.5 hours at 37°C. Following the
appropriate incubation times as described above, 10 μ l of each of the above
15 incubations were mixed with 10 μ l of 2X SDS-PAGE electrophoresis sample buffer,
and heated for 5 minutes in a boiling water bath. SDS-PAGE was performed
according to the method of Laemmli (Laemmli, Nature 227:680-685, 1970) using a
Mini-Protean II electrophoresis system with precast 4-15% Tris-Glycine
polyacrylamide gels, and under non-reducing conditions. Electrophoresis occurred at
20 200V for 45 minutes along with pre-stained molecular weight standards (Biorad).

After SDS-PAGE was performed as described above, the separated laminin
fragments were transferred to PVDF using a Mini transblot electrophoresis transfer
cell (Millipore, Bedford, MA, U.S.A.). Electrotransfer was performed at 100V for 2
hours. Following transfer, membranes were rinsed with methanol, dried and cut into
25 two equal parts which were used for A β ligand blotting, or Coomassie blue staining
and subsequent amino acid sequencing. The fragment(s) of laminin involved in
binding to A β were then detected by using biotinylated-A β (1-40), as described above.

Blots were probed for 2 hours with 2 μ M biotinylated A β (1-40) in TTBS. The membranes were then rinsed three times (10 seconds each) with TTBS, probed for 30 minutes with streptavidin alkaline phosphatase conjugate (Vectastain), rinsed again (as described above), and followed by the addition of an alkaline phosphatase
5 substrate solution (Vectastain). Following color development, the reaction was stopped by flushing the membranes with double distilled water.

For Coomassie blue staining, PVDF membranes were immersed with 0.2% Coomassie Brilliant blue (w/v) in 50 % methanol, 10% acetic acid, and 40% distilled water for 2 minutes, and then rinsed with 50% methanol, 10% acetic acid, and 40%
10 distilled water until visible bands were observed, and no background staining was present. The 55 kDa A β -binding laminin fragment, described below, was sent to the Biotechnology Service Center (Peptide Sequence Analysis Facility at the University of Toronto, Toronto, Ontario, Canada) and subjected to amino acid sequencing using a Porton 2090 Gas-Phase Microsequencer (Porton Instruments, Tarzana, CA) with
15 on-line analysis of phenylthiohydantoin derivatives.

In Figure 9, Panel A represents an A β ligand blot whereas Panel B represents the equivalent Coomassie blue stained blot. As shown in Figure 9 (Panel A, lanes 2 and 3), elastase-digested laminin produced multiple protein fragments which bound biotinylated A β (1-40). Panel A, lane 1 represents undigested mouse EHS laminin,
20 whereas lanes 2 and 3 represents laminin which had been digested with elastase for 1.5 hours or 2.5 hours, respectively. Panel A, lane 4 represents elastase digestion for 2.5 hours in the absence of laminin. Undigested laminin (Fig. 9, Panel A, lane 1) which interacted with A β included multiple bands from $> \sim 400$ kDa to $> \sim 86$ kDa, with the most prominent A β -interaction occurring with intact laminin (i.e. ~ 400 kDa).
25 Elastase-resistant laminin protein fragments which interacted with A β (Fig. 9, Panel A, lanes 2 and 3) included fragments of $> \sim 400$ kDa, ~ 130 kDa (arrowhead), ~ 80 -90 kDa, ~ 65 kDa and a prominent band at ~ 55 kDa (arrow). The interaction of these

elastase-resistant laminin protein fragments with A β were only observed under non-reducing conditions suggesting that the A β interaction was also conformation dependent. The 130kDa elastase resistant laminin fragment which interacts with A β , is also believed to be part of the E8 fragment (see Figure 11), and is the same protein
5 fragment of laminin that appears to be present in human serum and cerebrospinal fluid (see Examples 10 and 11). Figure 9, Panel A, lane 4 demonstrates that the band observed at ~29 kDa represents non-specific A β binding due to the presence of the elastase enzyme alone.

Figure 9, Panel B demonstrates all of the multiple protein bands which were
10 stained by Coomassie blue. Note, for example, in Panel B, lanes 2 and 3, that elastase digestion of laminin produced multiple protein fragments between ~55 kDa and ~90 kDa which did not bind A β , and were not observed in the A β ligand blot (Fig. 9, Panel A, lanes 2 and 3).

Example 7

15 **An A β -Binding Domain Within Laminin is Identified Within the Globular Repeats of the Laminin A Chain**

The 55 kDa laminin fragment (ie. produced following 1.5 hours of elastase digestion) that demonstrated positive A β binding interaction by ligand blotting was then prepared (Fig. 9, Panel B, lane 2, arrow) in large amounts for amino acid
20 sequencing (as described in example 6). Sequence data determined the exact location within laminin that was involved in binding to A β . An 11-amino acid sequence was determined from sequencing of the 55 kDa band. The sequence identified was:

Leu-His-Arg-Glu-His-Gly-Glu-Leu-Pro-Pro-Glu (SEQ ID NO:1)

The specific A β -binding domain within laminin was then identified by
25 comparison to known mouse laminin sequence (Sasaki and Yamada, J. Biol. Chem. 262:17111-17117, 1987; Sasaki et al, Proc. Natl. Acad. Sci. 84:935-939, 1987; Durkin, et al, Biochem. 27:5198-5204, 1988; Sasaki et al, J. Biol. Chem. 263:16536-16544,

1988), since mouse EHS laminin was utilized in the studies of the present invention. In addition, the complete amino acid sequence within laminin was retrieved from the National Center for Biotechnology Information, Bethesda, Maryland, U.S.A.

Figure 10 shows the complete amino acid sequence of mouse laminin A chain
5 (Genebank accession number P19137; SEQ ID NO: 4). The 11 amino acid protein fragment sequenced from the ~55 kDa protein within laminin which binds A β is identified (Figure 10; bold underline and arrowhead; SEQ ID NO: 1) and matches exactly to the region within the third globular domain repeat of laminin A chain (Figure 11). The fourth globular domain repeat of mouse laminin A chain is shown
10 as SEQ ID NO: 2 (Genebank Accession Number P19137; amino acids #2746-2922), whereas the fourth globular domain repeat of human laminin A chain is shown as SEQ ID NO: 3 (Genebank Accession Number P25391; amino acids #2737-2913).

Figure 11 shows two schematic representations of laminin (Colognato-Pyke et al, J. Biol. Chem. 270:9398-9406, 1995) and the newly discovered A β -binding region
15 of laminin (shown in left panel; between the two arrowheads) which is situated within the last three globular domains of the laminin A chain. The left panel of figure 11 illustrates laminin and fragments generated following protease digestions. Elastase fragments E1', E1X (dark line border), E-alpha-35 and E4 all correspond to regions of the short arms of laminin. Long arm fragments are E8, E3 and cathepsin G
20 fragment C8-9. The E8 fragment produced by elastase digestion of laminin contains the long arm fragments containing the distal part of the long arm and the G subdomains 1-3, and consists of a 130-150 kDa (Yurchenco and Cheng, J. Biol. Chem. 268:17286-17299, 1993). The E3 fragments also produced by elastase digestion of laminin contains the distal long arm globule with G subdomains 4 and 5. The E3
25 fragment shown in Figure 11, Panel A, has previously shown to be a doublet at ~60 kDa and ~55 kDa (Yurchenco and Cheng, J. Biol. Chem. 268:17286-17299, 1993). This

also confirms our discovery whereby the ~55 kDa fragment which we found to bind A β is localized within the E3 region of laminin (Figure 11, Left Panel).

The right panel of Figure 11 depicts the function map with the alpha (A chain), β (B1 chain) and gamma (B2 chain) chains of laminin shown in shades of decreasing darkness. EGF repeats are indicated by bars in the rod domains of the short arm. Domains, based on sequence analysis, are indicated in small Roman numerals and letters. The locations of heparin-binding, polymer-forming, and the active alpha1 β 1 integrin-binding sites are shown in bold-face for the alpha-chain short arm. The long arm functions of heparin binding (heparin), alpha6 β 1 integrin-recognition site (alpha6 β 1), and dystroglycan (DG), mapped in other studies, are indicated in gray-shaded labels. It is interesting to note that the A β -binding region of laminin is also a region involved in binding to heparin:

It should also be emphasized that the globular domain repeats of the laminin A chain likely interacts with A β in a conformation dependent manner, since the interaction of the ~55-kilodalton elastase-resistant protein fragments with A β was only observed under non-reducing conditions.

Example 8

Identification of Laminin and Laminin Protein Fragments in Human Serum and Cerebrospinal Fluid Derived from Alzheimer's disease, Type II Diabetes, and/or Normal Aged Patients

In the next study, western blotting techniques using a polyclonal antibody against laminin was used to determine whether intact laminin and/or laminin fragments were present in human serum and cerebrospinal fluid obtained from Alzheimer's disease, type II diabetes and/or normal aged patients. In this study, human serum was obtained from the Alzheimer's disease Research Center at the University of Washington from either living aged patients who may have had corresponding mini-mental state examinations (where a score of 30 is normal, a score

of 15 suggests moderate dementia and a score <10 suggests severe dementia), or from living aged patients who had subsequently died and were diagnosed at autopsy with Alzheimer's disease (following examination of their brains obtained postmortem). In addition, human serum was obtained from the Diabetes Endocrinology Research Center at the University of Washington. The following human serums were obtained and analyzed as part of this study: 1) patient #9; a normal 67 yr old female with a mini-mental score of 30; 2) patient #5226 - a 70 year old female with confirmed moderate Alzheimer's disease who also had a mini-mental score of 12 ; 3) patient #5211- a 66 year old male with confirmed Alzheimer's disease who also had a mini-mental score of 25; 4) patient B- a 63 year old male who had confirmed type II diabetes; 5) patient #5223- a 68 year old female with confirmed Alzheimer's disease who also had a mini-mental score of 22; 6) patient #22- an 83 yr old normal aged female who also had a mini-mental score of 30; 7) patient #C- a 68 year old male with confirmed type II diabetes. Each of these serums were utilized in this study and represent lanes 1-7 (left side) of Figure 12 (in the same order as above).

In addition, cerebrospinal fluid was obtained from the Alzheimer's disease Research Center at the University of Washington from either living aged patients who may have had corresponding mini-mental state examinations, or from living aged patients who had subsequently died and were diagnosed at autopsy with Alzheimer's disease (following examination of their brains obtained postmortem). The following human cerebrospinal fluids were obtained as part of this study: 1) patient #6- a normal 64 year old female who had a mini-mental score of 30; 2) patient #7- a normal 67 year old male who had a mini-mental score of 30; 3) patient #8- a normal 80 year old female who had a mini-mental score of 30; 4) patient #9- a normal 67 year old female who had a mini-mental score of 30; 5) patient #1111P- a normal 78 year old female who had a mini-mental score of 30; 6) patient #50- a 66 year old male patient with probable moderate Alzheimer's disease as indicated by a mini-mental score of

15; 7) patient #54-a 73 year old male with probable severe Alzheimer's disease as indicated by a mini-mental score of 8. Each of these cerebrospinal fluid samples were utilized in this study and represent lanes 1-7 (right side) of Figure 12 (in the same order as above).

5 For the study described above, 10 μ l of human serum diluted at 1:10, or 10 μ l of undiluted human cerebrospinal fluid was added to 10 μ l of SDS-PAGE buffer and ligand blots were prepared as in Example 6. Blots were probed for 2 hours with a polyclonal antibody (used at a dilution of 1:10,000 in TTBS) against EHS laminin (Sigma Chemical Company, St. Louis, MO). The membranes were then rinsed 3
10 times (10 seconds each) with TTBS and incubated for 1 hour with a biotinylated goat anti-rabbit IgG secondary antibody diluted 1:1,000 with TTBS. The membranes were then rinsed three times (10 seconds each) with TTBS, probed for 30 minutes with streptavidin alkaline phosphatase conjugate (Vectastain), rinsed again (as described above), followed by the addition of an alkaline phosphatase substrate solution
15 (Vectastain). Following color development, the reaction was stopped by flushing the membranes with double distilled water.

As shown in Figure 12, intact laminin (arrowheads) was present in human serum (lanes 1-7; left side) but not in human cerebrospinal fluid (lanes 1-7; right side). Qualitative observations suggest that intact laminin (as described above) may
20 have been decreased in serum of Alzheimer's disease patients in comparison to controls (i.e. compare intact laminin in Figure 12, lane 1, left side-normal individual; to Figure 12, lane 2, left side-Alzheimer's disease patient). In addition to intact laminin, human serum derived from Alzheimer's disease, type II diabetes and normal aged patients also contained laminin immunoreactivity in a series of band from ~120
25 kDa to ~200 kDa (Figure 12, bands observed between the two arrows). On the other hand, cerebrospinal fluid samples did not contain intact laminin (Figure 12; lanes 1-7; right side) but only contained a series of laminin immunoreactive protein fragments

from ~120 kDa to ~200 kDa (i.e. Figure 12, bands observed between the two arrows). This study determined that a series of laminin protein fragments are present in both human serum and cerebrospinal fluid of Alzheimer's disease, type II diabetes and normal aged patients, whereas intact laminin is only present in human serum. The
5 novel discovery of the laminin fragments in human cerebrospinal fluid suggests that it may be used as a marker to determine the extent of laminin breakdown in the brain during Alzheimer's disease and other brain disorders.

Example 9

Identification of a ~130 Kilodalton Laminin Protein Fragment in Human Serum of 10 Alzheimer's disease, Type II Diabetes and Normal Aged Patients which Binds A β

In the next study, A β ligand blotting techniques were utilized to identify whether laminin or laminin protein fragments present in human serum bind A β . In this study, human serum was obtained from the Alzheimer's disease Research Center at the University of Washington from either living patients who may have had
15 corresponding mini-mental state examinations (where a score of 30 is normal, a score of 15 suggests moderate dementia and a score <10 suggests severe dementia), or from living patients who had subsequently died and were diagnosed at autopsy with Alzheimer's disease (following examination of their brains obtained postmortem). In addition, human serum was obtained from the Diabetes Endocrinology Research
20 Center at the University of Washington. The first six human serum samples (i.e. Figure 13, lanes 1-6) were the same serum samples as indicated in Example 8. In addition, Figure 13 lanes 7-10 consisted of human serum obtained from lane 7) patient #E- a 54 year old male with confirmed type II diabetes, lane 8) patient #5230- a 72 year old female with confirmed moderate Alzheimer's disease who had a
25 mini-mental score of 19, lane 9) patient #E-a 54 year old male with confirmed type II diabetes, and lane 10) patient #F- a 69 year old male with confirmed type II diabetes.

For this study, A β (1-40) was biotinylated according to the manufacturer's protocol (Pierce, Rockford, IL). For the ligand studies, following SDS-PAGE as described above in Example 8, separated laminin and its fragments present in human serum were transferred to polyvinylidene difluoride membrane (PVDF) using a Mini
5 transblot electrophoresis transfer cell. Electrotransfer was performed at 100V for 2 hours. Following transfer, membranes were rinsed with methanol and dried. The fragment(s) of laminin in human serum involved in binding to A β were then detected by using biotinylated-A β (1-40). Blots were probed for 2 hours with 1 μ M biotinylated A β (1-40) in TTBS. The membranes were then rinsed three times (10 seconds each)
10 with TTBS, probed for 30 minutes with streptavidin alkaline phosphatase conjugate (Vectastain), rinsed again (as described above), and followed by the addition of an alkaline phosphatase substrate solution (Vectastain). Following color development, the reaction was stopped by flushing the membranes with double distilled water.

As shown in Figure 13, A β interacted with intact human laminin (arrow) in
15 most samples of human serum. However, it was surprising to note that intact laminin was virtually absent in 2 of the 4 Alzheimer's disease patients serum (Fig. 13, lanes 5 and 8), suggesting that laminin-derived fragments may be important in Alzheimer's disease as a diagnostic marker. The most interesting discovery was that of all the laminin immunoreactive protein fragments found in human serum (i.e ~120
20 kDa to ~200 kDa, bands observed between the arrows, Figure 12, lanes 1-7, right side), only a prominent ~130 kDa band was found to interact with A β (Figure 13, arrowhead). This same prominent band is approximately the same molecular weight of the E8 band generated from mouse laminin following elastase digestion (see Figure 9), and which also contains the globular domain repeats of the laminin A chain. This
25 study therefore determined that besides intact laminin, human serum contains a ~130 kDa laminin fragment which binds to A β , and may be important for keeping A β soluble in biological fluids such as blood. This study also suggests that qualitative

and quantitative assessment of laminin fragments in human serum may prove diagnostic for the extent and progression of Alzheimer's disease, type II diabetes and other amyloidoses.

5

Example 10

Identification of a ~130 Kilodalton Laminin Protein Fragment in Human Cerebrospinal Fluid of Alzheimer's disease and Normal Aged Patients which Binds A β

In the next study, A β ligand blotting techniques were utilized to identify whether laminin protein fragments (<200 kDa) present in human cerebrospinal fluid bind A β . In this study, human cerebrospinal fluid was obtained from the Alzheimer's disease Research Center at the University of Washington from either living aged patients who may have had corresponding mini-mental state examinations (where a score of 30 is normal, a score of 15 suggests moderate Alzheimer's disease and a score <10 suggests moderate Alzheimer's disease), or from living aged patients who had subsequently died and were diagnosed at autopsy with Alzheimer's disease (following examination of their brains obtained postmortem). The following human cerebrospinal fluids were obtained and analyzed as part of this study (depicted in Figure 14, lanes 1-10): 1) patient #65- a 71 yr old male with probable severe Alzheimer's disease as indicated by a mini-mental score of 0; 2) patient #54-a 73 yr old male with probable severe Alzheimer's disease as indicated by a mini-mental score of 8.; 3) patient #6- a normal 64 yr old female who had a mini-mental score of 30; 4) patient #7- a normal 67 yr old male who had a mini-mental score of 30; 5) patient #8- a normal 80 yr old female who had a mini-mental score of 30; 6) patient #9- a normal 67 yr old female who had a mini-mental score of 30; 7) patient #1111P- a normal 78 yr old female who had a mini-mental score of 30; 8) patient #50-a 66 yr old male patient with probable moderate Alzheimer's disease as indicated by a mini-mental score of 15; 9) patient #52-a 69 yr old male with probable moderate Alzheimer's

disease as indicated by a mini-mental score of 16; 10) patient #64-a 64 yr old male with probable severe Alzheimer's disease as indicated by a mini-mental score of 0. Each of these cerebrospinal fluid samples were utilized in this study and represent lanes 1-10 of Figure 14 (in the same order as above).

5 For this study, A β ligand blotting was employed as described in Example 9. The fragment(s) of laminin in human cerebrospinal fluid involved in binding to A β were detected by using biotinylated-A β (1-40). Blots were probed for 2 hours with 50 nM of biotinylated A β (1-40) in TTBS. The rest of the A β ligand blotting procedure is as described above in Example 9.

10 As shown in Figure 14, A β interacted with laminin fragment bands between ~120 kDa and ~200 kDa in most samples of human cerebrospinal fluid. As observed in human serum, most samples of human cerebrospinal fluid also contained a prominent ~130 kDa laminin fragment (Figure 14, arrow) which interacted with A β . No intact A β -binding laminin was found in human cerebrospinal fluid (not shown), as previously
15 demonstrated (Figure 12, Example 8). Again, this same prominent ~130 kDa A β -binding laminin fragment present in human cerebrospinal fluid is approximately the same molecular weight of the E8 band generated from laminin, and which also contains the globular domain repeats of the laminin A chain. This study therefore determined that human cerebrospinal fluid also contains a ~130 kDa laminin fragment
20 which binds to A β , and may be important for keeping A β soluble in biological fluids such as cerebrospinal fluid.

Further Aspects and Utilizations of the Invention

Laminin-Derived Protein Fragments and Polypeptides

One therapeutic application of the present invention is to use laminin, laminin protein fragments which bind A β or other amyloid proteins, and/or laminin polypeptides derived from amino acid sequencing of the laminin fragments which bind A β (such as the ~130 kilodalton protein described herein) or other amyloid proteins, as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses. The amyloid diseases include, but are not limited to, the amyloid associated with Alzheimer's disease and Down's syndrome (wherein the specific amyloid is referred to as beta-amyloid protein or A β), the amyloid associated with chronic inflammation, various forms of malignancy and Familial Mediterranean Fever (wherein the specific amyloid is referred to as AA amyloid or inflammation-associated amyloidosis), the amyloid associated with multiple myeloma and other B-cell dyscrasias (wherein the specific amyloid is referred to as AL amyloid), the amyloid associated with type II diabetes (wherein the specific amyloid is referred to as amylin or islet amyloid), the amyloid associated with the prion diseases including Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, kuru and animal scrapie (wherein the specific amyloid is referred to as PrP amyloid), the amyloid associated with long-term hemodialysis and carpal tunnel syndrome (wherein the specific amyloid is referred to as beta₂-microglobulin amyloid), the amyloid associated with senile cardiac amyloid and Familial Amyloidotic Polyneuropathy (wherein the specific amyloid is referred to as transthyretin or prealbumin), and the amyloid associated with endocrine tumors such as medullary carcinoma of the thyroid (wherein the specific amyloid is referred to as variants of procalcitonin).

The polypeptides referred to above may be a natural polypeptide, a synthetic polypeptide or a recombinant polypeptide. The fragments, derivatives or analogs of the polypeptides to any laminin fragment referred to herein may be a) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue and such substituted amino acid residue may or may not be encoded by the genetic code, or b) one in which one or more of the amino acid residues includes a substituent group, or c) one in which the mature polypeptide is fused with another compound, such as a compound used to increase the half-life of the polypeptide (for example, polylysine), or d) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of the invention.

The tertiary structure of proteins refers to the overall 3-dimensional architecture of a polypeptide chain. The complexity of 3-dimensional structure arises from the intrinsic ability of single covalent bonds to be rotated. Rotation about several such bonds in a linear molecule will produce different nonsuperimposable 3-dimensional arrangements of the atoms that are generally described as conformations.

Protein conformation is an essential component of protein-protein, protein-substrate, protein-agonist, protein-antagonist interactions. Changes in the component amino acids of protein sequences can result in changes that have little or no effect on the resultant protein conformation. Conversely, changes in the peptide sequences can have effects on the protein conformation resulting in reduced or increased protein-protein, etc. interactions. Such changes and their effects are generally disclosed in Proteins: Structures and Molecular Properties by Thomas

Creightonm W.H. Freeman and Company, New York, 1984 which is hereby incorporated by reference.

“Conformation” and “conformation similarity” when used in this specification and claims refers to a polypeptide’s ability (or any other organic or inorganic molecule) to assume a given shape, through folding and the like, so that the shape, or conformation, of the molecule becomes an essential part of it’s functionality, sometimes to the exclusion of its chemical makeup. It is generally known that in biological processes two conformational similar molecules may be interchangeable in the process, even the chemically different. “Conformational similarity” refers to the latter interchangeability or substitutability. For example, laminin and laminin-derived protein fragments are among the subjects of the invention because they have been shown to bind the A β protein and render it inactive in fibril formation; it is contemplated that other molecules that are conformationally similar to laminin, or any claimed laminin fragment or polypeptide, may be substituted in the claimed method to similarly render the A β inactive in fibrillogenesis and other amyloid processes. In general it is contemplated that levels of conformational similarity at or above 70% are sufficient to assume homologous functionality in the claimed processes, though reduced levels of conformational similarity may be made to serve as well. Conformational similar levels at or above 90% should provide some level of additional homologue functionality.

Thus, one skilled in the art would envisage that changes can be made to the laminin sequence, or fragments or polypeptides thereof, that would increase, decrease or have no effect on the binding of laminin or fragments thereof, to A β amyloid. In addition, one skilled in the art would envisage various post-translational modifications such as phosphorylation, glycosylation and the like would alter the binding of laminin, laminin fragments or laminin polypeptides to A β amyloid.

The polypeptides of the present invention include the polypeptides or fragments of laminin described herein, including but not limited to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and
5 fragments thereof, as well as polypeptides which have at least 70% similarity (preferably 70 % identity) and more preferably a 90% similarity (more preferably a 90% identity) to the polypeptides described above.

Fragments or portions of the polypeptides or fragments of laminin of the present invention may be employed for producing the corresponding full-length
10 polypeptides by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full length polypeptides.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant,
15 insect and mammalian cells in culture). Depending upon the host employed in a recombinant procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

Chemical polypeptide synthesis is a rapidly evolving area in the art, and
20 methods of solid phase polypeptide synthesis are well-described in the following references, hereby entirely incorporated by reference (Merrifield, J.Amer.Chem.Soc. 85:2149-2154, 1963; Merrifield, Science 232:341-347, 1986; Fields, Int.J.Polypeptide Prot.Res. 35, 161, 1990).

Recombinant production of laminin polypeptides can be accomplished according
25 to known method steps. Standard reference works setting forth the general principles of recombinant DNA technology include Watson, Molecular Biology of the Gene, Volumes I and II, The Benjamin/Cummings Publishing Company Inc., publisher,

Menlo Park, Calif. 1987; Ausubel et al, eds., Current Protocols in Molecular Biology, Wiley Interscience, publisher, New York, N.Y. 1987; 1992; and Sambrook et al, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, publisher, Cold Spring Harbor, N.Y. 1989, the entire contents of which
5 references are herein incorporated by reference.

The polypeptides of the present invention may also be utilized as research reagents and materials for discovery of treatments and diagnostics for human diseases.

Antibodies

10 Antibodies generated against the polypeptides corresponding to specific sequences recognizing the laminin fragments of the present invention which bind A β or other amyloid proteins can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even
15 a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptides from tissue expressing that polypeptide. Preferred embodiments include, but are not limited to, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8,
20 SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, and fragments thereof, as well as polypeptides which have at least 70% similarity (preferably 70 % identity) and more preferably a 90% similarity (more preferably a 90% identity) to the polypeptides described above.

The term "antibody" is meant to include polyclonal antibodies, monoclonal
25 antibodies, chimeric antibodies, anti-idiotypic antibodies to antibodies specific for laminin-derived protein fragments or polypeptides of the present invention.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen.

5 A monoclonal antibody contains a substantially homogeneous population of antibodies specific to antigens, which population contains substantially similar epitope binding sites. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, Nature 256:495-497, 1975), the trioma technique, the human B-cell hybridoma technique (Kozbor et al, Immunology Today 4:72, 1983), and the EBV-hybridoma technique to produce human
10 monoclonal antibodies (Cole et al, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp.77-96, 1985). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, GILD and any subclass thereof.

Chimeric antibodies are molecules different portions of which are derived from different animal species, such as those having variable region derived from a murine
15 monoclonal antibody and a human immunoglobulin constant region, which are primarily used to reduce immunogenicity in application and to increase yields in production. Chimeric antibodies and methods for their production are known in the art (ex. Cabilly et al, Proc.Natl.Acad.Sci.U.S.A 81:3273-3277, 1984; Harlow and Lane: Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory 1988).

20 An anti-idiotypic antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An anti-idiotypic antibody can be prepared by immunizing an animal of the same species and genetic type (e.g., mouse strain) as the source of the monoclonal antibody with the monoclonal antibody to which an anti-idiotypic antibody is being prepared. The
25 immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the

anti-idiotypic antibody). See, for example, U.S. Patent No. 4,699,880, which is herein incorporated by reference.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and F(ab')₂, which are capable of binding antigen. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear
5 more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al, J. Nucl. Med. 24:316-325, 1983).

The antibodies or fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect laminin or laminin-derived fragments
10 in a sample or to detect presence of cells which express a laminin polypeptide of the present invention. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric or fluorometric detection.

One of the ways in which a laminin fragment antibody can be detectably
15 labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA). This enzyme, in turn, when later exposed to an appropriate substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric, or by visual means. Enzymes which can be used detectably label the antibody include, but are not limited
20 to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. The detection can be
25 accomplished by colometric methods which employ a chromogenic substrate for the enzyme. Detection can be accomplished by colometric methods which employ a chromogenic substrate for the enzyme. Detection can also be accomplished by visual

comparison of the extent of enzymatic reaction of a substrate with similarly prepared standards (see Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory 1988; Ausubel et al, eds., Current Protocols in Molecular Biology, Wiley Interscience, N.Y. 1987, 1992).

5 Detection may be accomplished using any of a variety of other immunoassays. For example, by radiolabeling of the antibodies or antibody fragments, it is possible to detect R-PTPase through the use of a radioimmunoassay (RIA). A good description of RIA may be found in Laboratory Techniques and Biochemistry in Molecular Biology, by Work et al, North Holland Publishing Company, NY (1978) with particular
10 reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, incorporated entirely by reference herein. The radioactive isotope can be detected by such means as the use of a gamma-counter, a scintillation counter or by autoradiography.

 It is also possible to label a laminin fragment polypeptide antibody with a
15 fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labelling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine, commercially available, e.g., from Molecular
20 Probes, Inc. (Eugene, Oregon, U.S.A.).

 The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu , or other of the lanthanide series. These metals can be attached to the antibody using such metal groups as diethylenetriamine pentaacetic acid (EDTA).

 The antibody can also be detectably labeled by coupling it to a
25 chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent

labeling compounds are luminol, isoluminol, thermotropic acridinium ester, imidazole, acridinium salt, and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

The antibodies (or fragments thereof) useful in the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for in situ detection of a laminin fragment of the present invention. In situ detection may be accomplished by removing a histological specimen from a patient, and providing the labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of a laminin fragment polypeptide but also its distribution on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

In accordance with yet a further aspect of the present invention there are provided antibodies against laminin, laminin fragments and/or laminin-derived polypeptides which interact with A β or other amyloid proteins, or derivatives thereof. These antibodies can be used for a number of important diagnostic and/or therapeutic applications as described herein. In one aspect of the invention, polyclonal and/or monoclonal antibodies made against laminin, laminin fragments and/or laminin-derived polypeptides which bind A β or other amyloid proteins, may be

utilized for Western blot analysis (using standard Western blotting techniques knowledgeable to those skilled in the art) to detect the presence of amyloid protein-binding laminin fragments or amyloid protein-binding laminin polypeptides in human tissues and in tissues of other species. Western blot analysis can also be
5 used to determine the apparent size of each amyloid protein-binding laminin fragment. In addition, Western blotting following by scanning densitometry (known to those skilled in the art) can be used to quantitate and compare levels of each of the laminin fragments in tissue samples, biological fluids or biopsies obtained from individuals with specific diseases (such as the amyloid diseases) in comparison to
10 tissue samples, biological fluids or biopsies obtained from normal individuals or controls. Biological fluids, include, but are not limited to, blood, plasma, serum, cerebrospinal fluid, sputum, saliva, urine and stool.

In yet another aspect of the invention, polyclonal and/or monoclonal antibodies made against laminin, laminin fragments and/or laminin-derived peptides which bind
15 A β or other amyloid proteins, can be utilized for immunoprecipitation studies (using standard immunoprecipitation techniques known to one skilled in the art) to detect laminin, laminin fragments and/or laminin-derived peptides which bind A β or other amyloid proteins, in tissues, cells and/or biological fluids. Use of the laminin, laminin fragment and/or laminin-derived peptide antibodies for immunoprecipitation studies
20 can also be quantitated to determine relative levels of laminin, laminin fragments and/or laminin-derived peptides which interact with A β or other amyloid proteins, in tissues, cells and/or biological fluids. Quantitative immunoprecipitation can be used to compare levels of laminin, laminin fragments and/or laminin amyloid protein-binding peptides in tissue samples, biological fluids or biopsies obtained from
25 individuals with specific diseases (such as the amyloid diseases) in comparison to tissue samples, biological fluids or biopsies obtained from normal individuals or controls.

Therapeutic Applications

Yet another aspect of the present invention is to make use of laminin, laminin fragments and/or laminin-derived polypeptides as amyloid inhibitory therapeutic agents. The laminin-derived peptide sequences or fragments can be synthesized
5 utilizing standard techniques (ie. using an automated synthesizer). Laminin, laminin fragments and/or laminin-derived polypeptides which bind A β or other amyloid proteins, can be used as potential blocking therapeutics for the interaction of laminin in a number of biological processes and diseases (such as in the amyloid diseases described above). In a preferred embodiment, specific peptides made against the
10 amino acid sequence of laminin contained within the ~55 kDa laminin fragment (i.e. globular repeats within the laminin A chain; SEQ ID NO 3) described in the present invention, may be used to aid in the inhibition of amyloid formation, deposition, accumulation, and /or persistence in a given patient. Likewise, in another preferred
15 embodiment anti-idiotypic antibodies made against laminin, laminin fragments and/or laminin-derived polypeptides (as described above) may be given to a human patient as potential blocking antibodies to disrupt continued amyloid formation, deposition, accumulation and/or persistence in the given patient.

Preparations of laminin-derived polypeptides for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which
20 may contain axillary agents or excipients which are known in the art. Pharmaceutical compositions such as tablets, pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, syrups, tea bags, aerosols (as a solid or in a liquid medium), suppositories, sterile injectable solutions, sterile packaged powders, can be
25 prepared according to routine methods and are known in the art.

In yet another aspect of the invention, laminin, laminin fragments and/or laminin-derived polypeptides may be used as an effective therapy to block amyloid

formation, deposition, accumulation and/or persistence as observed in the amyloid diseases. For example, the invention includes a pharmaceutical composition for use in the treatment of amyloidoses comprising a pharmaceutically effective amount of a laminin, laminin fragment and/or laminin-derived polypeptide anti-idiotypic antibody and a pharmaceutically acceptable carrier. The compositions may contain the laminin, laminin fragments and/or laminin-derived polypeptide anti-idiotypic antibody, either unmodified, conjugated to a potentially therapeutic compound, conjugated to a second protein or protein portion or in a recombinant form (ie. chimeric or bispecific laminin, laminin fragment and/or laminin polypeptide antibody).

The compositions may additionally include other antibodies or conjugates. The antibody compositions of the invention can be administered using conventional modes of administration including, but not limited to, topical, intravenous, intra-arterial, intraperitoneal, oral, intralymphatic, intramuscular or intralumbar. Intravenous administration is preferred. The compositions of the invention can be a variety of dosage forms, with the preferred form depending upon the mode of administration and the therapeutic application. Optimal dosage and modes of administration for an individual patient can readily be determined by conventional protocols.

Laminin, laminin-derived protein fragments, and laminin-derived polypeptides, or antibodies of the present invention may be administered by any means that achieve their intended purpose, for example, to treat laminin involved pathologies, such as Alzheimer's disease and other amyloid diseases, or other related pathologies, using a laminin-derived polypeptide described herein, in the form of a pharmaceutical composition.

For example, administration of such a composition may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal or buccal routes. Alternatively, or

concurrently, administration may be by the oral route. Parenteral administration can be by bolus injection or by gradual perfusion over time.

A preferred mode of using a laminin-derived polypeptide; or antibody pharmaceutical composition of the present invention is by oral administration or
5 intravenous application.

A typical regimen for preventing, suppressing or treating laminin-involved pathologies, such as Alzheimer's disease amyloidosis, comprises administration of an effective amount of laminin-derived polypeptides, administered over a period of one or several days, up to and including between one week and about 24 months.

10 It is understood that the dosage of the laminin-derived polypeptides of the present invention administered in vivo or in vitro will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill
15 in the art, without undue experimentation.

The total dose required for each treatment may be administered by multiple doses or in a single dose. A laminin-derived polypeptide may be administered alone or in conjunction with other therapeutics directed to laminin-involved pathologies, such as Alzheimer's disease or amyloid diseases, as described herein.

20 Effective amounts of a laminin-derived polypeptide or composition, which may also include a laminin-fragment derived antibody, are about 0.01 μ g to about 100mg/kg body weight, and preferably from about 10 μ g to about 50 mg/kg body weight, such as 0.05, 0.07, 0.09, 0.1, 0.5, 0.7, 0.9., 1, 2, 5, 10, 20, 25, 30, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 mg/kg.

25 Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain axillary agents or excipients which are known in the art. Pharmaceutical compositions

comprising at least one laminin-derived polypeptide, such as 1-10 or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 laminin-derived polypeptides, of the present invention may include all compositions wherein the laminin-derived polypeptide is contained in an amount effective to achieve its intended purpose. In addition to at least one laminin-derived polypeptide, a pharmaceutical composition may contain suitable pharmaceutically acceptable carriers, such as excipients, carriers and/or auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically.

Pharmaceutical compositions comprising at least one laminin-derived polypeptide or antibody may also include suitable solutions for administration intravenously, subcutaneously, dermally, orally, mucosally, rectally or may by injection or orally, and contain from about 0.01 to 99 percent, preferably about 20 to 75 percent of active component (i.e. polypeptide or antibody) together with the excipient. Pharmaceutical compositions for oral administration include pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixers, suspensions, emulsions, solutions, and syrups.

The laminin, laminin-derived protein fragments, and laminin-derived polypeptides for Alzheimer's disease and other central nervous system amyloidoses may be optimized to cross the blood-brain barrier. Methods of introductions include but are not limited to systemic administration, parenteral administration i.e., via an intraperitoneal, intravenous, perioral, subcutaneous, intramuscular, intraarterial, intradermal, intramuscular, intranasal, epidural and oral routes. In a preferred embodiment, laminin, laminin-derived protein fragments, and laminin-derived polypeptides may be directly administered to the cerebrospinal fluid by intraventricular injection. In a specific embodiment, it may be desirable to administer laminin, laminin-derived protein fragments, and laminin-derived polypeptides locally to the area or tissue in need of treatment; this may be achieved by, for example, and

not by way of limitation, local infusion during surgery, topical application, by injection, by infusion using a cannulae with osmotic pump, by means of a catheter, by means of a suppository, or by means of an implant.

In yet another embodiment laminin, laminin-derived protein fragments, and
5 laminin-derived polypeptides may be delivered in a controlled release system, such as an osmotic pump. In yet another embodiment, a controlled release system can be placed in proximity to the therapeutic target, ie. the brain, thus requiring only a fraction of the systemic dose.

In yet another aspect of the present invention, peptidomimetic compounds
10 modelled from laminin, laminin fragments and/or laminin-derived polypeptides identified as binding A β or other amyloid proteins, may serve as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses. Peptidomimetic modelling is implemented by standard procedures known to those skilled in the art.

15 In yet another aspect of the present invention, compounds that mimic the 3-dimensional A β binding site on laminin using computer modelling, may serve as potent inhibitors of amyloid formation, deposition, accumulation and/or persistence in Alzheimer's disease and other amyloidoses. Design and production of such compounds using computer modelling technologies is implemented by standard
20 procedures known to those skilled in the art.

Recombinant DNA technology, including human gene therapy, has direct applicability to the laminin proteins and their fragments, of this invention. One skilled in the art can take the peptide sequences disclosed herein and create corresponding nucleotide sequences that code for the corresponding peptide sequences.
25 These sequences can be cloned into vectors such as retroviral vectors, and the like. These vectors can, in turn, be transfected into human cells such as hepatocytes or fibroblasts, and the like. Such transfected cells can be introduced into humans to

treat amyloid diseases. Alternatively, the genes can be introduced into the patients directly. The basic techniques of recombinant DNA technology are known to those of ordinary skill in the art and are disclosed in Recombinant DNA Second Edition, Watson, et al., W.H. Freeman and Company, New York, 1992, which is hereby
5 incorporated by reference.

Diagnostic Applications

Another aspect of the invention is to provide polyclonal and/or monoclonal antibodies against laminin, laminin fragments and/or laminin-derived polypeptides which bind A β or other amyloid proteins, which would be utilized to specifically detect
10 laminin, laminin fragments and/or laminin-derived peptides in human tissues and/or biological fluids. In one preferred embodiment, polyclonal or monoclonal antibodies made against a peptide portion or fragment of laminin, can be used to detect and quantify laminin, laminin fragments and/or laminin-derived polypeptides in human tissues and/or biological fluids. Polyclonal and/or monoclonal peptide antibodies can
15 also be utilized to specifically detect laminin fragments and/or laminin-derived polypeptides in human tissues and/or biological fluids. In a preferred embodiment, a polyclonal or monoclonal antibody made specifically against a peptide portion or fragment of ~55 kDa elastase-resistant protein which binds A β (as described herein), can be used to detect and quantify this laminin fragment in human tissues and/or
20 biological fluids. In another preferred embodiment, a polyclonal or monoclonal antibody made specifically against a peptide portion or fragment of ~130 kDa laminin-derived protein which is present in human biological fluids and binds A β (as described herein), can be used to detect and quantify this laminin fragment in human tissues and/or biological fluids. Other preferred embodiments include, but are not
25 limited to, making polyclonal or monoclonal antibodies made specifically against a peptide portion or fragment of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9,

SEQ ID NO: 10 and SEQ ID NO: 11, as well as polypeptides which have at least 70% similarity (preferably 70 % identity) and more preferably a 90% similarity (more preferably a 90% identity) to the polypeptides described above.

For detection of laminin fragments and/or laminin-derived polypeptides
5 described above in human tissues, cells, and/or in cell culture, the polyclonal and/or monoclonal antibodies can be utilized using standard immunohistochemical and immunocytochemical techniques, known to one skilled in the art.

For detection and quantitation of laminin, laminin fragments and/or laminin-derived polypeptides in biological fluids, including cerebrospinal fluid, blood,
10 plasma, serum, urine, sputum, and/or stool, various types of ELISA assays can be utilized, known to one skilled in the art. An antibody molecule of the present invention may be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support or carrier,
15 and a quantity of detectable labeled soluble antibody is added to permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

In a preferred embodiment, a "sandwich" type of ELISA can be used. Using this preferred method a pilot study is first implemented to determine the quantity of
20 binding of each laminin-fragment monoclonal antibody to microtiter wells. Once this is determined, aliquots (usually in 40 µl of TBS; pH 7.4) of the specific laminin-fragment antibody are allowed to bind overnight to microtiter wells (Maxisorb C plate from Nunc) at 4°C. A series of blank wells not containing any laminin-fragment specific monoclonal antibody are also utilized as controls. The next
25 day, non-bound monoclonal antibody is shaken off the microtiter wells. All of the microtiter wells (including the blank wells) are then blocked by incubating for 2 hours with 300 µl of Tris-buffered saline containing 0.05% Tween-20 (TTBS) plus 2% bovine

serum albumin, followed by 5 rinses with TTBS. 200 µl of cerebrospinal fluid, blood, plasma, serum, urine, sputum, and/or stool and/or any other type of biological sample is then diluted (to be determined empirically) in TTBS containing 2% bovine serum albumin and placed in wells (in triplicate) containing bound laminin-fragment
5 antibody (or blank) and incubated for 2 hours at room temperature. The wells are then washed 5 times with TTBS. A second biotinylated-monoclonal antibody against the same laminin-derived fragment (but which is against a different epitope) is then added to each well (usually in 40 µl of TBS; pH 7.4) and allowed to bind for 2 hours at room temperature to any laminin-fragment captured by the first antibody.
10 Following incubation, the wells are washed 5 times with TTBS. Bound materials are then detected by incubating with 100 µl of peroxidase-avidin complex (1:250 dilution in TTBS with 0.1% BSA) for 1 hour on a rotary shaker. After 5 washes with TTBS, a substrate solution (100 µl, OPD-Sigma Fast from Sigma Chemical Co., St. Louis, MO, USA) is added and allowed to develop significant color (usually 8-10 minutes).
15 The reaction is stopped with 50 µl of 4N sulfuric acid and read on a standard spectrophotometer at 490 nm. This ELISA can be utilized to determine differences in specific laminin fragments (and/or Aβ-binding laminin fragments) in biological fluids which can serve as a diagnostic marker to follow the progression on a live patient during the progression of disease (ie. monitoring of amyloid disease as an
20 example). In addition, quantitative changes in laminin fragments can also serve as a prognostic indicator monitoring how a live patient will respond to treatment which targets a given amyloid disease such as Alzheimer's disease. Such assays can be provided in a kit form.

A competition assay may also be employed wherein antibodies specific to
25 laminin, laminin fragments and/or laminin-derived polypeptides are attached to a solid support and labelled laminin, laminin fragments and/or laminin-derived polypeptides and a sample derived from a host are passed over the solid support and

the amount of label detected attached to the solid support can be correlated to the quantity of laminin, laminin fragments and/or laminin-derived polypeptides in the sample. This standard technique is known to one skilled in the art.

Another object of the present invention is to use laminin, laminin fragments
5 and/or laminin-derived polypeptides, in conjunction with laminin, laminin fragment and/or laminin-derived peptide antibodies, in an ELISA assay to detect potential laminin, laminin fragment and/or laminin-derived peptide autoantibodies in human biological fluids. Such a diagnostic assay may be produced in a kit form. In a preferred embodiment, peptides containing the sequences of laminin, laminin-derived
10 fragments and laminin-derived polypeptides as in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, as well as polypeptides which have at least 70% similarity (preferably 70 % identity) and more preferably a 90% similarity (more preferably a 90% identity) to the polypeptides described above, will
15 be used to initially bind to microtiter wells in an ELISA plate. A pilot study is first implemented to determine the quantity of binding of each laminin fragment polypeptide to microtiter wells. Once this is determined, aliquots (usually 1-2 μ g in 40 μ l of TBS; pH 7.4) of specific laminin fragment polypeptides (as described herein) are allowed to bind overnight to microtiter wells (Maxisorb C plate from Nunc) at 4°C.
20 All the microtiter wells (including blank wells without the laminin fragment polypeptides) are blocked by incubating for 2 hours with 300 μ l of Tris-buffered saline (pH 7.4) with 0.05% Tween-20 (TTBS), containing 2% albumin. This is followed by 5 rinses with TTBS. The patients' biological fluids (i.e., cerebrospinal fluid, blood, plasma, serum, sputum, urine, and/or stool) are then utilized and 200 μ l are diluted
25 (to be determined empirically) with TTBS containing 2% bovine serum albumin, and placed in microtiter wells (in triplicate) containing a specific laminin fragment polypeptide or blank wells (which do not contain peptide), and are incubated at 1.5

hours at room temperature. Any autoantibodies present in the biological fluids against the laminin fragment will bind to the substrate bound laminin fragment polypeptide (or fragments thereof). The wells are then rinsed by washing 5 times with TTBS. 100 μ l of biotinylated polyclonal goat anti-human IgGs (Sigma Chemical company, St. Louis, MO, USA), diluted 1:500 in TTBS with 0.1% bovine serum albumin, is then aliquoted into each well. Bound materials are detected by incubating with 100 μ l of peroxidase-avidin complex (1:250 dilution in TTBS with 0.1% bovine serum albumin) for 1 hour on a rotary shaker. Following 5 washes with TTBS, substrate solution (100 μ l, OPD-Sigma Fast from Sigma Chemical Company, St. Louis, MO, USA) is added and allowed to develop significant color (usually 8-10 minutes). The reaction is stopped with 50 μ l of 4N sulfuric acid added to each well and read on a standard spectrophotometer at 490 nm. This assay system can be utilized to not only detect the presence of autoantibodies against laminin fragments in biological fluids, but also to monitor the progression of disease by following elevation or diminution of laminin fragment autoantibody levels. It is believed that patients demonstrating excessive laminin fragment formation, deposition, accumulation and/or persistence as may be observed in the amyloid diseases, will also carry autoantibodies against the laminin fragments in their biological fluids. Various ELISA assay systems, knowledgeable to those skilled in the art, can be used to accurately monitor the degree of laminin fragments in biological fluids as a potential diagnostic indicator and prognostic marker for patients during the progression of disease (ie. monitoring of an amyloid disease for example). Such assays can be provided in a kit form. In addition, quantitative changes in laminin fragment autoantibody levels can also serve as a prognostic indicator monitoring how a live patient will respond to treatment which targets a given amyloid disease.

Other diagnostic methods utilizing the invention include diagnostic assays for measuring altered levels of laminin, laminin fragments and/or laminin-derived

polypeptides in various tissues compared to normal control tissue samples. Assays used to detect levels of laminin, laminin fragments and/or laminin-derived polypeptides in a sample derived from a host are well-known to those skilled in the art and included radioimmunoassays, competitive-binding assays, Western blot
5 analysis and preferably ELISA assays (as described above).

Yet another aspect of the present invention is to use the antibodies recognizing laminin, laminin fragments and/or laminin-derived polypeptides for labellings, for example, with a radionucleotide, for radioimaging or radioguided surgery, for in vivo diagnosis, and/or for in vitro diagnosis. In one preferred embodiment, radiolabelled
10 peptides or antibodies made (by one skilled in the art) against laminin, laminin fragments and/or laminin-derived polypeptides may be used as minimally invasive techniques to locate laminin, laminin fragments and/or laminin-derived polypeptides, and concurrent amyloid deposits in a living patient. These same imaging techniques could then be used at regular intervals (ie. every 6 months) to monitor the
15 progression of the amyloid disease by following the specific levels of laminin, laminin fragments and/or laminin-derived polypeptides.

Yet another aspect of the present invention is to provide a method which can evaluate a compound's ability to alter (diminish or eliminate) the affinity of a given amyloid protein (as described herein) or amyloid precursor protein, to laminin,
20 laminin-derived fragments or laminin-derived polypeptides. By providing a method of identifying compounds which affect the binding of amyloid proteins, or amyloid precursor proteins to such laminin-derived fragments, the present invention is also useful in identifying compounds which can prevent or impair such binding interaction. Thus, compounds can be identified which specifically affect an event linked with the
25 amyloid formation, amyloid deposition, and/or amyloid persistence condition associated with Alzheimer's disease and other amyloid diseases as described herein.

According to one aspect of the invention, to identify for compounds which allow the interaction of amyloid proteins or precursor proteins to laminin-derived fragments or laminin polypeptides, either amyloid or laminin fragments are immobilized, and the other of the two is maintained as a free entity. The free entity is contacted with
5 the immobilized entity in the presence of a test compound for a period of time sufficient to allow binding of the free entity to the immobilized entity, after which the unbound free entity is removed. Using antibodies which recognize the free entity, or other means to detect the presence of bound components, the amount of free entity bound to immobilized entity can be measured. By performing this assay in the
10 presence of a series of known concentrations of test compound and, as a control, the complete absence of test compound, the effectiveness of the test compound to allow binding of free entity to immobilized entity can be determined and a quantitative determination of the effect of the test compound on the affinity of free entity to immobilized entity can be made. By comparing the binding affinity of the
15 amyloid-laminin fragment complex in the presence of a test compound to the binding affinity of the amyloid-laminin fragment complex in the absence of a test compound, the ability of the test compound to modulate the binding can be determined.

In the case in which the amyloid is immobilized, it is contacted with free laminin-derived fragments or polypeptides, in the presence of a series of
20 concentrations of test compound. As a control, immobilized amyloid is contacted with free laminin-derived polypeptides, or fragments thereof in the absence of the test compound. Using a series of concentrations of laminin-derived polypeptides, the dissociation constant (K_d) or other indicators of binding affinity of amyloid-laminin fragment binding can be determined. In the assay, after the laminin-derived
25 polypeptides or fragments thereof is placed in contact with the immobilized amyloid for a sufficient time to allow binding, the unbound laminin polypeptides are removed. Subsequently, the level of laminin fragment-amyloid binding can be observed. One

method uses laminin-derived fragment antibodies, as described in the invention, to detect the amount of specific laminin fragments bound to the amyloid or the amount of free laminin fragments remaining in solution. This information is used to determine first qualitatively whether or not the test compound can allow continued binding
5 between laminin-derived fragments and amyloid. Secondly, the data collected from assays performed using a series of test compounds at various concentrations, can be used to measure quantitatively the binding affinity of the laminin fragment-amyloid complex and thereby determine the effect of the test compound on the affinity between laminin fragments and amyloid. Using this information, compounds can be
10 identified which do not modulate the binding of specific laminin fragments to amyloid and thereby allow the laminin-fragments to reduce the amyloid formation, deposition, accumulation and/or persistence, and the subsequent development and persistence of amyloidosis.

Therefore a kit for practicing a method for identifying compounds useful which
15 do not alter laminin, laminin-derived fragments or laminin-derived polypeptides to an immobilized amyloid protein, said kit comprising a) a first container having amyloid protein immobilized upon the inner surface, b) a second container which contains laminin, laminin-derived fragments or laminin-derived polypeptides dissolved in solution, c) a third container which contains antibodies specific for said laminin,
20 laminin-derived fragments or laminin-derived polypeptides, said antibodies dissolved in solution, and d) a fourth container which contains labelled antibodies specific for laminin, laminin-derived fragments or laminin-derived polypeptides, said antibodies dissolved in solution.

In compliance with the statute, the invention has been described in language
25 more or less specific as to structural features. It is to be understood, however, that the invention is not limited to the specific features shown, since the means and

construction shown comprise preferred forms of putting the invention into effect. The invention is, therefore, claimed in any of its forms or modifications within the legitimate and valid scope of the appended claims, appropriately interpreted in accordance with the doctrine of equivalents.

SEQUENCE LISTING

(I) GENERAL INFORMATION

(I) APPLICANTS: Gerardo Castillo and Alan Snow

(ii) TITLE OF APPLICATION: Therapeutic and Diagnostic Applications of Laminin and Laminin-Derived Protein Fragments

(iii) NUMBER OF SEQUENCES: 11

INFORMATION FOR SEQ ID NO: 1:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 11 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P19137

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 1:

Leu His Arg Glu His Gly Glu Leu Pro Pro Glu
5 10

INFORMATION FOR SEQ ID NO: 2:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 177 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P19137 (AMINO ACIDS #2746-2922)

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 2:

Leu	Gln	Val	Gln	Leu	Ser	Ile	Arg	Thr	Phe	Ala	Ser	Ser	Gly	Leu	Ile	Tyr	Tyr	Val	Ala	1	5	10	15	20
His	Gln	Asn	Gln	Met	Asp	Tyr	Ala	Thr	Leu	Gln	Leu	Gln	Glu	Gly	Arg	Leu	His	Phe	Met	25	30	35	40	
Phe	Asp	Leu	Gly	Lys	Gly	Arg	Thr	Lys	Val	Ser	His	Pro	Ala	Leu	Leu	Ser	Asp	Gly	Lys	45	50	55	60	
Trp	His	Thr	Val	Lys	Thr	Glu	Tyr	Ile	Lys	Arg	Lys	Ala	Phe	Met	Thr	Val	Asp	Gly	Gln	65	70	75	80	
Glu	Ser	Pro	Ser	Val	Thr	Val	Val	Gly	Asn	Ala	Thr	Thr	Leu	Asp	Val	Glu	Arg	Lys	Leu	85	90	95	100	
Tyr	Leu	Gly	Gly	Leu	Pro	Ser	His	Tyr	Arg	Ala	Arg	Asn	Ile	Gly	Thr	Ile	Thr	His	Ser	105	110	115	120	
Ile	Pro	Ala	Cys	Ile	Gly	Glu	Ile	Met	Val	Asn	Gly	Gln	Gln	Leu	Asp	Lys	Asp	Arg	Pro	125	130	135	140	
Leu	Ser	Ala	Ser	Ala	Val	Asp	Arg	Cys	Tyr	Val	Val	Ala	Gln	Glu	Gly	Thr	Phe	Phe	Glu	145	150	155	160	
Gly	Ser	Gly	Tyr	Ala	Ala	Leu	Val	Lys	Glu	Gly	Tyr	Lys	Val	Arg	Leu	Asp				165	170	175		

INFORMATION FOR SEQ ID NO: 3:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 177 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P25391 (AMINO ACIDS #2737-2913)

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 3:

```

Leu Ser Val Glu Leu Ser Ile Arg Thr Phe Ala Ser Ser Gly Leu Ile Tyr Tyr Met Ala
 1           5           10          15          20
His Gln Asn Gln Ala Asp Tyr Ala Val Leu Gln Leu His Gly Gly Arg Leu His Phe Met
          25          30          35          40
Phe Asp Leu Gly Lys Gly Arg Thr Lys Val Ser His Pro Ala Leu Leu Ser Asp Gly Lys
          45          50          55          60
Trp His Thr Val Lys Thr Asp Tyr Val Lys Arg Lys Gly Phe Ile Thr Val Asp Gly Arg
          65          70          75          80
Glu Ser Pro Met Val Thr Val Val Gly Asp Gly Thr Met Leu Asp Val Glu Gly Leu Phe
          85          90          95          100
Tyr Leu Gly Gly Leu Pro Ser Gln Tyr Gln Ala Arg Lys Ile Gly Asn Ile Thr His Ser
          105         110         115         120
Ile Pro Ala Cys Ile Gly Asp Val Thr Val Asn Ser Lys Gln Leu Asp Lys Asp Ser Pro
          125         130         135         140
Val Ser Ala Phe Thr Val Asn Arg Cys Tyr Ala Val Ala Gln Glu Gly Thr Tyr Phe Asp
          145         150         155         160
Gly Ser Gly Tyr Ala Ala Leu Val Lys Glu Gly Tyr Lys Val Gln Ser Asp
          165         170         175

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INFORMATION FOR SEQ ID NO: 4:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 3084 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P19137

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 4:

Met	Arg	Gly	Ser	Gly	Thr	Gly	Ala	Ala	Leu	Leu	Val	Leu	Leu	Ala	Ser	Val	Leu	Trp	Val	1	5	10	15	20
Thr	Val	Arg	Ser	Gln	Gln	Arg	Gly	Leu	Phe	Pro	Ala	Ile	Leu	Asn	Leu	Ala	Thr	Asn	Ala	25	30	35	40	
His	Ile	Ser	Ala	Asn	Ala	Thr	Cys	Gly	Glu	Lys	Gly	Pro	Glu	Met	Phe	Cys	Lys	Leu	Val	45	50	55	60	
Glu	His	Val	Pro	Gly	Arg	Pro	Val	Arg	His	Ala	Gln	Cys	Arg	Val	Cys	Asp	Gly	Asn	Ser	65	70	75	80	
Thr	Asn	Pro	Arg	Glu	Arg	His	Pro	Ile	Ser	His	Ala	Ile	Asp	Gly	Thr	Asn	Asn	Trp	Trp	85	90	95	100	
Gln	Ser	Pro	Ser	Ile	Gln	Asn	Gly	Arg	Glu	Tyr	His	Trp	Val	Thr	Val	Thr	Leu	Asp	Leu	105	110	115	120	
Arg	Gln	Val	Phe	Gln	Val	Ala	Tyr	Ile	Ile	Ile	Lys	Ala	Ala	Asn	Ala	Pro	Arg	Pro	Gly	125	130	135	140	
Asn	Trp	Ile	Leu	Glu	Arg	Ser	Val	Asp	Gly	Val	Lys	Phe	Lys	Pro	Trp	Gln	Tyr	Tyr	Ala	145	150	155	160	
Val	Ser	Asp	Thr	Glu	Cys	Leu	Thr	Arg	Tyr	Lys	Ile	Thr	Pro	Arg	Arg	Gly	Pro	Pro	Thr	165	170	175	180	
Tyr	Arg	Ala	Asp	Asn	Glu	Val	Ile	Cys	Thr	Ser	Tyr	Tyr	Ser	Lys	Leu	Val	Pro	Leu	Glu	185	190	195	200	
His	Gly	Glu	Ile	His	Thr	Ser	Leu	Ile	Asn	Gly	Arg	Pro	Ser	Ala	Asp	Asp	Pro	Ser	Pro	205	210	215	220	
Gln	Leu	Leu	Glu	Phe	Thr	Ser	Ala	Arg	Tyr	Ile	Arg	Leu	Arg	Leu	Gln	Arg	Ile	Arg	Thr	225	230	235	240	
Leu	Asn	Ala	Asp	Leu	Met	Thr	Leu	Ser	His	Arg	Asp	Leu	Arg	Asp	Leu	Asp	Pro	Ile	Val	245	250	255	260	
Thr	Arg	Arg	Tyr	Tyr	Tyr	Ser	Ile	Lys	Asp	Ile	Ser	Val	Gly	Gly	Met	Cys	Ile	Cys	Tyr	265	270	275	280	
Gly	His	Ala	Ser	Ser	Cys	Pro	Trp	Asp	Glu	Glu	Ala	Lys	Gln	Leu	Gln	Cys	Gln	Cys	Glu	285	290	295	300	
His	Asn	Thr	Cys	Gly	Glu	Ser	Cys	Asp	Arg	Cys	Cys	Pro	Gly	Tyr	His	Gln	Gln	Pro	Trp	305	310	315	320	
Arg	Pro	Gly	Thr	Ile	Ser	Ser	Gly	Asn	Glu	Cys	Glu	Glu	Cys	Asn	Cys	His	Asn	Lys	Ala	325	330	335	340	
Lys	Asp	Cys	Tyr	Tyr	Asp	Ser	Ser	Val	Ala	Lys	Glu	Arg	Arg	Ser	Leu	Asn	Thr	Ala	Gly	345	350	355	360	
Gln	Tyr	Ser	Gly	Gly	Gly	Val	Cys	Val	Asn	Cys	Ser	Gln	Asn	Thr	Thr	Gly	Ile	Asn	Cys	365	370	375	380	
Glu	Thr	Cys	Ile	Asp	Gln	Tyr	Tyr	Arg	Pro	His	Lys	Val	Ser	Pro	Tyr	Asp	Asp	His	Pro	385	390	395	400	
Cys	Arg	Pro	Cys	Asn	Cys	Asp	Pro	Val	Gly	Ser	Leu	Ser	Ser	Val	Cys	Ile	Lys	Asp	Asp	405	410	415	420	

Arg His Ala Asp	Leu Ala Asn Gly Lys	Trp Pro Gly Gln Cys	Pro Cys Arg Lys Gly Tyr
425	430	435	440
Ala Gly Asp Lys	Cys Asp Arg Cys Gln	Phe Gly Tyr Arg Gly	Phe Pro Asn Cys Ile Pro
445	450	455	460
Cys Asp Cys Arg	Thr Val Gly Ser Leu	Asn Glu Asp Pro Cys	Ile Glu Pro Cys Leu Cys
465	470	475	480
Lys Lys Asn Val	Glu Gly Lys Asn Cys	Asp Arg Cys Lys Pro	Gly Phe Tyr Asn Leu Lys
485	490	495	500
Glu Arg Asn Pro	Glu Gly Cys Ser Glu	Cys Phe Cys Phe Gly	Val Ser Gly Val Cys Asp
505	510	515	520
Ser Leu Thr Trp	Ser Ile Ser Gln Val	Thr Asn Met Ser Gly	Trp Leu Val Thr Asp Leu
525	530	535	540
Met Ser Thr Asn	Lys Ile Arg Ser Gln	Gln Asp Val Leu Gly	Gly His Arg Gln Ile Ser
545	550	555	560
Ile Asn Asn Thr	Ala Val Met Gln Arg	Leu Thr Ser Thr Tyr	Tyr Trp Ala Ala Pro Glu
565	570	575	580
Ala Tyr Leu Gly	Asn Lys Leu Thr Ala	Phe Gly Gly Phe Leu	Lys Tyr Thr Val Ser Tyr
585	590	595	600
Asp Ile Pro Val	Glu Thr Val Asp Ser	Asp Leu Met Ser His	Ala Asp Ile Ile Ile Lys
605	610	615	620
Gly Asn Gly Leu	Thr Ile Ser Thr Arg	Ala Glu Gly Leu Ser	Leu Gln Pro Tyr Glu Glu
625	630	635	640
Tyr Phe Asn Val	Val Arg Leu Val Pro	Glu Asn Phe Arg Asp	Phe Asn Thr Arg Arg Glu
645	650	655	660
Ile Asp Arg Asp	Gln Leu Met Thr Val	Leu Ala Asn Val Thr	His Leu Leu Ile Arg Ala
665	670	675	680
Asn Tyr Asn Ser	Ala Lys Met Ala Leu	Tyr Arg Leu Asp Ser	Val Ser Leu Asp Ile Ala
685	690	695	700
Ser Pro Asn Ala	Ile Asp Leu Ala Val	Ala Ala Asp Val Glu	His Cys Glu Cys Pro Gln
705	710	715	720
Gly Tyr Thr Gly	Thr Ser Cys Glu Ala	Cys Leu Pro Gly Tyr	Tyr Arg Val Asp Gly Ile
725	730	735	740
Leu Phe Gly Gly	Ile Cys Gln Pro Cys	Glu Cys His Gly His	Ala Ser Glu Cys Asp Ile
745	750	755	760
His Gly Ile Cys	Ser Val Cys Thr His	Asn Thr Thr Gly Asp	His Cys Glu Gln Cys Leu
765	770	775	780
Pro Gly Phe Tyr	Gly Thr Pro Ser Arg	Gly Thr Pro Gly Asp	Cys Gln Pro Cys Ala Cys
785	790	795	800
Pro Leu Ser Ile	Asp Ser Asn Asn Phe	Ser Pro Thr Cys His	Leu Thr Asp Gly Glu Glu
805	810	815	820
Val Val Cys Asp	Gln Cys Ala Pro Gly	Tyr Ser Gly Ser Trp	Cys Glu Arg Cys Ala Asp
825	830	835	840
Gly Tyr Tyr Gly	Asn Pro Thr Val Pro	Gly Gly Thr Cys Val	Pro Cys Asn Cys Ser Gly
845	850	855	860
Asn Val Asp Pro	Leu Glu Ala Gly His	Cys Asp Ser Val Thr	Gly Glu Cys Leu Lys Cys
865	870	875	880
Leu Trp Asn Thr	Asp Gly Ala His Cys	Glu Arg Cys Ala Asp	Gly Phe Tyr Gly Asp Ala
885	890	895	900
Val Thr Ala Lys	Asn Cys Arg Ala Cys	Asp Cys His Glu Asn	Gly Ser Leu Ser Gly Val
905	910	915	920
Cys His Leu Glu	Thr Gly Leu Cys Asp	Cys Lys Pro His Val	Thr Gly Gln Gln Cys Asp
925	930	935	940
Gln Cys Leu Ser	Gly Tyr Tyr Gly Leu	Asp Thr Gly Leu Gly	Cys Val Pro Cys Asn Cys
945	950	955	960

Ser Val Glu Gly	Ser Val Ser Asp Asn Cys Thr Glu Glu Gly	Gln Cys His Cys Gly	Pro
965	970	975	980
Gly Val Ser Gly	Lys Gln Cys Asp Arg Cys Ser His Gly Phe Tyr Ala Phe Gln Asp Gly		
985	990	995	1000
Gly Cys Thr Pro	Cys Asp Cys Ala His Thr Gln Asn Asn Cys Asp	Pro Ala Ser Gly	Glu
1005	1010	1015	1020
Cys Leu Cys Pro	Pro His Thr Gln Gly Leu Lys Cys Glu Glu Cys Glu Glu Ala Tyr Trp		
1025	1030	1035	1040
Gly Leu Asp Pro	Glu Gln Gly Cys Gln Ala Cys Asn Cys Ser Ala Val Gly Ser Thr Ser		
1045	1050	1055	1060
Ala Gln Cys Asp	Val Leu Ser Gly His Cys Pro Cys Lys Lys Gly Phe Gly Gly Gln Ser		
1065	1070	1075	1080
Cys His Gln Cys	Ser Leu Gly Tyr Arg Ser Phe Pro Asp Cys Val Pro Cys Gly Cys Asp		
1085	1090	1095	1100
Leu Arg Gly Thr	Leu Pro Asp Thr Cys Asp Leu Glu Gln Gly Leu Cys Ser Cys Ser Glu		
1105	1110	1115	1120
Asp Ser Gly Thr	Cys Ser Cys Lys Glu Asn Val Val Gly Pro Gln Cys Ser Lys Cys Gln		
1125	1130	1135	1140
Ala Gly Thr Phe	Ala Leu Arg Gly Asp Asn Pro Gln Gly Cys Ser Pro Cys Phe Cys Phe		
1145	1150	1155	1160
Gly Leu Ser Gln	Leu Cys Ser Glu Leu Glu Gly Tyr Val Arg Thr Leu Ile Thr Leu Ala		
1165	1170	1175	1180
Ser Asp Gln Pro	Leu Leu His Val Val Ser Gln Ser Asn Leu Lys Gly Thr Ile Glu Gly		
1185	1190	1195	1200
Val His Phe Gln	Pro Pro Asp Thr Leu Leu Asp Ala Glu Ala Val Arg Gln His Ile Tyr		
1205	1210	1215	1220
Ala Glu Pro Phe	Tyr Trp Arg Leu Pro Lys Gln Phe Gln Gly Asp Gln Leu Leu Ala Tyr		
1225	1230	1235	1240
Gly Gly Lys Leu	Gln Tyr Ser Val Ala Phe Tyr Ser Thr Leu Gly Thr Gly Thr Ser Asn		
1245	1250	1255	1260
Tyr Glu Pro Gln	Val Leu Ile Lys Gly Gly Arg Ala Arg Lys His Val Ile Tyr Met Asp		
1265	1270	1275	1280
Ala Pro Ala Pro	Glu Asn Gly Val Arg Gln Asp Tyr Glu Val Gln Met Lys Glu Glu Phe		
1285	1290	1295	1300
Trp Lys Tyr Phe	Asn Ser Val Ser Glu Lys His Val Thr His Ser Asp Phe Met Ser Val		
1305	1310	1315	1320
Leu Ser Asn Ile	Asp Tyr Ile Leu Ile Lys Ala Ser Tyr Gly Gln Gly Leu Gln Gln Ser		
1325	1330	1335	1340
Arg Ile Ala Asn	Ile Ser Met Glu Val Gly Arg Lys Ala Val Glu Leu Pro Ala Glu Gly		
1345	1350	1355	1360
Glu Ala Ala Leu	Leu Leu Glu Leu Cys Val Cys Pro Pro Gly Thr Ala Gly His Ser Cys		
1365	1370	1375	1380
Gln Asp Cys Ala	Pro Gly Tyr Tyr Arg Glu Lys Leu Pro Glu Ser Gly Gly Arg Gly Pro		
1385	1390	1395	1400
Arg Pro Leu Leu	Ala Pro Cys Val Pro Cys Asn Cys Asn Asn His Ser Asp Val Cys Asp		
1405	1410	1415	1420
Pro Glu Thr Gly	Lys Cys Leu Ser Cys Arg Asp His Thr Ser Gly Asp His Cys Glu Leu		
1425	1430	1435	1440
Cys Ala Ser Gly	Tyr Tyr Gly Lys Val Thr Gly Leu Pro Gly Asp Cys Thr Pro Cys Thr		
1445	1450	1455	1460
Cys Pro His His	Pro Pro Phe Ser Phe Ser Pro Thr Cys Val Val Glu Gly Asp Ser Asp		
1465	1470	1475	1480
Phe Arg Cys Asn	Ala Cys Leu Pro Gly Tyr Glu Gly Gln Tyr Cys Glu Arg Cys Ser Ala		
1485	1490	1495	1500

Gly Tyr His Gly Asn Pro Arg Ala Ala Gly Gly Ser Cys Gln Thr Cys Asp Cys Asn Pro	1505	1510	1515	1520
Gln Gly Ser Val His Ser Asp Cys Asp Arg Ala Ser Gly Gln Cys Val Cys Lys Pro Gly	1525	1530	1535	1540
Ala Thr Gly Leu His Cys Glu Lys Cys Leu Pro Arg His Ile Leu Met Glu Ser Asp Cys	1545	1550	1555	1560
Val Ser Cys Asp Asp Asp Cys Val Gly Pro Leu Leu Asn Asp Leu Asp Ser Val Gly Asp	1565	1570	1575	1580
Ala Val Leu Ser Leu Asn Leu Thr Gly Val Ser Pro Ala Pro Tyr Gly Ile Leu Glu Asn	1585	1590	1595	1600
Leu Glu Asn Thr Thr Lys Tyr Phe Gln Arg Tyr Leu Ile Lys Glu Asn Ala Lys Lys Ile	1605	1610	1615	1620
Arg Ala Glu Ile Gln Leu Glu Gly Ile Ala Glu Gln Thr Glu Asn Leu Gln Lys Glu Leu	1625	1630	1635	1640
Thr Arg Val Leu Ala Arg His Gln Lys Val Asn Ala Glu Met Glu Arg Thr Ser Asn Gly	1645	1650	1655	1660
Thr Gln Ala Leu Ala Thr Phe Ile Glu Gln Leu His Ala Asn Ile Lys Glu Ile Thr Glu	1665	1670	1675	1680
Lys Val Ala Thr Leu Asn Gln Thr Ala Arg Lys Asp Phe Gln Pro Pro Val Ser Ala Leu	1685	1690	1695	1700
Gln Ser Met His Gln Asn Ile Ser Ser Leu Leu Gly Leu Ile Lys Glu Arg Asn Phe Thr	1705	1710	1715	1720
Glu Met Gln Gln Asn Ala Thr Leu Glu Leu Lys Ala Ala Lys Asp Leu Leu Ser Arg Ile	1725	1730	1735	1740
Gln Lys Arg Phe Gln Lys Pro Gln Glu Lys Leu Lys Ala Leu Lys Glu Ala Asn Ser Leu	1745	1750	1755	1760
Leu Ser Asn His Ser Glu Lys Leu Gln Ala Ala Glu Glu Leu Leu Lys Glu Ala Gly Ser	1765	1770	1775	1780
Lys Thr Gln Glu Ser Asn Leu Leu Leu Leu Leu Val Lys Ala Asn Leu Lys Glu Glu Phe	1785	1790	1795	1800
Gln Glu Lys Lys Leu Arg Val Gln Glu Glu Gln Asn Val Thr Ser Glu Leu Ile Ala Lys	1805	1810	1815	1820
Gly Arg Glu Trp Val Asp Ala Ala Gly Thr His Thr Ala Ala Ala Gln Asp Thr Leu Thr	1825	1830	1835	1840
Gln Leu Glu His His Arg Asp Glu Leu Leu Leu Trp Ala Arg Lys Ile Arg Ser His Val	1845	1850	1855	1860
Asp Asp Leu Val Met Gln Met Ser Lys Arg Arg Ala Arg Asp Leu Val His Arg Ala Glu	1865	1870	1875	1880
Gln His Ala Ser Glu Leu Gln Ser Arg Ala Gly Ala Leu Asp Arg Asp Leu Glu Asn Val	1885	1890	1895	1900
Arg Asn Val Ser Leu Asn Ala Thr Ser Ala Ala His Val His Ser Asn Ile Gln Thr Leu	1905	1910	1915	1920
Thr Glu Glu Ala Glu Met Leu Ala Ala Asp Ala His Lys Thr Ala Asn Lys Thr Asp Leu	1925	1930	1935	1940
Ile Ser Glu Ser Leu Ala Ser Arg Gly Lys Ala Val Leu Gln Arg Ser Ser Arg Phe Leu	1945	1950	1955	1960
Lys Glu Ser Val Gly Thr Arg Arg Lys Gln Gln Gly Ile Thr Met Lys Leu Asp Glu Leu	1965	1970	1975	1980
Lys Asn Leu Thr Ser Gln Phe Gln Glu Ser Val Asp Asn Ile Thr Lys Gln Ala Asn Asp	1985	1990	1995	2000
Ser Leu Ala Met Leu Arg Glu Ser Pro Gly Gly Met Arg Glu Lys Gly Arg Lys Ala Arg	2005	2010	2015	2020
Glu Leu Ala Ala Ala Ala Asn Glu Ser Ala Val Lys Thr Leu Glu Asp Val Leu Ala Leu	2025	2030	2035	2040

Ser Leu Arg Val Phe Asn Thr Ser Glu Asp Leu Ser Arg Val Asn Ala Thr Val Gln Glu
 2045 2050 2055 2060
 Thr Asn Asp Leu Leu His Asn Ser Thr Met Thr Thr Leu Leu Ala Gly Arg Lys Met Lys
 2065 2070 2075 2080
 Asp Met Glu Met Gln Ala Asn Leu Leu Leu Asp Arg Leu Lys Pro Leu Lys Thr Leu Glu
 2085 2090 2095 2100
 Glu Asn Leu Ser Arg Asn Leu Ser Glu Ile Lys Leu Leu Ile Ser Arg Ala Arg Lys Gln
 2105 2110 2115 2120
 Ala Ala Ser Ile Lys Val Ala Val Ser Ala Asp Arg Asp Cys Ile Arg Ala Tyr Gln Pro
 2125 2130 2135 2140
 Gln Thr Ser Ser Thr Asn Tyr Asn Thr Leu Ile Leu Asn Val Lys Thr Gln Glu Pro Asp
 2145 2150 2155 2160
 Asn Leu Leu Phe Tyr Leu Gly Ser Ser Ser Ser Ser Asp Phe Leu Ala Val Glu Met Arg
 2165 2170 2175 2180
 Arg Gly Lys Val Ala Phe Leu Trp Asp Leu Gly Ser Gly Ser Thr Arg Leu Glu Phe Pro
 2185 2190 2195 2200
 Glu Val Ser Ile Asn Asn Asn Arg Trp His Ser Ile Tyr Ile Thr Arg Phe Gly Asn Met
 2205 2210 2215 2220
 Gly Ser Leu Ser Val Lys Glu Ala Ser Ala Ala Glu Asn Pro Pro Val Arg Thr Ser Lys
 2225 2230 2235 2240
 Ser Pro Gly Pro Ser Lys Val Leu Asp Ile Asn Asn Ser Thr Leu Met Phe Val Gly Gly
 2245 2250 2255 2260
 Leu Gly Gly Gln Ile Lys Lys Ser Pro Ala Val Lys Val Thr His Phe Lys Gly Cys Met
 2265 2270 2275 2280
 Gly Glu Ala Phe Leu Asn Gly Lys Ser Ile Gly Leu Trp Asn Tyr Ile Glu Arg Glu Gly
 2285 2290 2295 2300
 Lys Cys Asn Gly Cys Phe Gly Ser Ser Gln Asn Glu Asp Ser Ser Phe His Phe Asp Gly
 2305 2310 2315 2320
 Ser Gly Tyr Ala Met Val Glu Lys Thr Leu Arg Pro Thr Val Thr Gln Ile Val Ile Leu
 2325 2330 2335 2340
 Phe Ser Thr Phe Ser Pro Asn Gly Leu Leu Phe Tyr Leu Ala Ser Asn Gly Thr Lys Asp
 2345 2350 2355 2360
 Phe Leu Ser Ile Glu Leu Val Arg Gly Arg Val Lys Val Met Val Asp Leu Gly Ser Gly
 2365 2370 2375 2380
 Pro Leu Thr Leu Met Thr Asp Arg Arg Tyr Asn Asn Gly Thr Trp Tyr Lys Ile Ala Phe
 2385 2390 2395 2400
 Gln Arg Asn Arg Lys Gln Gly Leu Leu Ala Val Phe Asp Ala Tyr Asp Thr Ser Asp Lys
 2405 2410 2415 2420
 Glu Thr Lys Gln Gly Glu Thr Pro Gly Ala Ala Ser Asp Leu Asn Arg Leu Glu Lys Asp
 2425 2430 2435 2440
 Leu Ile Tyr Val Gly Gly Leu Pro His Ser Lys Ala Val Arg Lys Gly Val Ser Ser Arg
 2445 2450 2455 2460
 Ser Tyr Val Gly Cys Ile Lys Asn Leu Glu Ile Ser Arg Ser Thr Phe Asp Leu Leu Arg
 2465 2470 2475 2480
 Asn Ser Tyr Gly Val Arg Lys Gly Cys Ala Leu Glu Pro Ile Gln Ser Val Ser Phe Leu
 2485 2490 2495 2500
 Arg Gly Gly Tyr Val Glu Met Pro Pro Lys Ser Leu Ser Pro Glu Ser Ser Leu Leu Ala
 2505 2510 2515 2520
 Thr Phe Ala Thr Lys Asn Ser Ser Gly Ile Leu Leu Val Ala Leu Gly Lys Asp Ala Glu
 2525 2530 2535 2540
 Glu Ala Gly Gly Ala Gln Ala His Val Pro Phe Phe Ser Ile Met Leu Leu Glu Gly Arg
 2545 2550 2555 2560
 Ile Glu Val His Val Asn Ser Gly Asp Gly Thr Ser Leu Arg Lys Ala Leu Leu His Ala
 2565 2570 2575 2580

Pro Thr Gly Ser Tyr Ser Asp Gly Gln Glu His Ser Ile Ser Leu Val Arg Asn Arg Arg
 2585 2590 2595 2600
 Val Ile Thr Ile Gln Val Asp Glu Asn Ser Pro Val Glu Met Lys Leu Gly Pro Leu Thr
 2605 2610 2615 2620
 Glu Gly Lys Thr Ile Asp Ile Ser Asn Leu Tyr Ile Gly Gly Leu Pro Glu Asp Lys Ala
 2625 2630 2635 2640
 Thr Pro Met Leu Lys Met Arg Thr Ser Phe His Gly Cys Ile Lys Asn Val Val Leu Asp
 2645 2650 2655 2660
 Ala Gln Leu Leu Asp Phe Thr His Ala Thr Gly Ser Glu Gln Val Glu Leu Asp Thr Cys
 2665 2670 2675 2680
 Leu Leu Ala Glu Glu Pro Met Gln Ser Leu His Arg Glu His Gly Glu Leu Pro Pro Glu
 2685 2690 2695 2700
 Pro Pro Thr Leu Pro Gln Pro Glu Leu Cys Ala Val Asp Thr Ala Pro Gly Tyr Val Ala
 2705 2710 2715 2720
 Gly Ala His Gln Phe Gly Leu Ser Gln Asn Ser His Leu Val Leu Pro Leu Asn Gln Ser
 2725 2730 2735 2740
 Asp Val Arg Lys Arg Leu Gln Val Gln Leu Ser Ile Arg Thr Phe Ala Ser Ser Gly Leu
 2745 2750 2755 2760
 Ile Tyr Tyr Val Ala His Gln Asn Gln Met Asp Tyr Ala Thr Leu Gln Leu Gln Glu Gly
 2765 2770 2775 2780
 Arg Leu His Phe Met Phe Asp Leu Gly Lys Gly Arg Thr Lys Val Ser His Pro Ala Leu
 2785 2790 2795 2800
 Leu Ser Asp Gly Lys Trp His Thr Val Lys Thr Glu Tyr Ile Lys Arg Lys Ala Phe Met
 2805 2810 2815 2820
 Thr Val Asp Gly Gln Glu Ser Pro Ser Val Thr Val Val Gly Asn Ala Thr Thr Leu Asp
 2825 2830 2835 2840
 Val Glu Arg Lys Leu Tyr Leu Gly Gly Leu Pro Ser His Tyr Arg Ala Arg Asn Ile Gly
 2845 2850 2855 2860
 Thr Ile Thr His Ser Ile Pro Ala Cys Ile Gly Glu Ile Met Val Asn Gly Gln Gln Leu
 2865 2870 2875 2880
 Asp Lys Asp Arg Pro Leu Ser Ala Ser Ala Val Asp Arg Cys Tyr Val Val Ala Gln Glu
 2885 2890 2895 2900
 Gly Thr Phe Phe Glu Gly Ser Gly Tyr Ala Ala Leu Val Lys Glu Gly Tyr Lys Val Arg
 2905 2910 2915 2920
 Leu Asp Leu Asn Ile Thr Leu Glu Phe Arg Thr Thr Ser Lys Asn Gly Val Leu Leu Gly
 2925 2930 2935 2940
 Ile Ser Ser Ala Lys Val Asp Ala Ile Gly Leu Glu Ile Val Asp Gly Lys Val Leu Phe
 2945 2950 2955 2960
 His Val Asn Asn Gly Ala Gly Arg Ile Thr Ala Thr Tyr Gln Pro Arg Ala Ala Arg Ala
 2965 2970 2975 2980
 Leu Cys Asp Gly Lys Trp His Thr Leu Gln Ala His Lys Ser Lys His Arg Ile Val Leu
 2985 2990 2995 3000
 Thr Val Asp Gly Asn Ser Val Arg Ala Glu Ser Pro His Thr His Ser Thr Ser Ala Asp
 3005 3010 3015 3020
 Thr Asn Asp Pro Ile Tyr Val Gly Gly Tyr Pro Ala His Ile Lys Gln Asn Cys Leu Ser
 3025 3030 3035 3040
 Ser Arg Ala Ser Phe Arg Gly Cys Val Arg Asn Leu Arg Leu Ser Arg Gly Ser Gln Val
 3045 3050 3055 3060
 Gln Ser Leu Asp Leu Ser Arg Ala Phe Asp Leu Gln Gly Val Phe Pro His Ser Cys Pro
 3065 3070 3075 3080
 Gly Pro Glu Pro

INFORMATION FOR SEQ ID NO: 5:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 3075 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P25391

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 5:

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Met Arg Gly Gly Val Leu Leu Val Leu Leu Leu Cys Val Ala Ala Gln Cys Arg Gln Arg
 1          5          10          15          20
Gly Leu Phe Pro Ala Ile Leu Asn Leu Ala Ser Asn Ala His Ile Ser Thr Asn Ala Thr
 25          30          35          40
Cys Gly Glu Lys Gly Pro Glu Met Phe Cys Lys Leu Val Glu His Val Pro Gly Arg Pro
 45          50          55          60
Val Arg Asn Pro Gln Cys Arg Ile Cys Asp Gly Asn Ser Ala Asn Pro Arg Glu Arg His
 65          70          75          80
Pro Ile Ser His Ala Ile Asp Gly Thr Asn Asn Trp Trp Gln Ser Pro Ser Ile Gln Asn
 85          90          95          100
Gly Arg Glu Tyr His Trp Val Thr Ile Thr Leu Asp Leu Arg Gln Val Phe Gln Val Ala
 105         110         115         120
Tyr Val Ile Ile Lys Ala Ala Asn Ala Pro Arg Pro Gly Asn Trp Ile Leu Glu Arg Ser
 125         130         135         140
Leu Asp Gly Thr Thr Phe Ser Pro Trp Gln Tyr Tyr Ala Val Ser Asp Ser Glu Cys Leu
 145         150         155         160
Ser Arg Tyr Asn Ile Thr Pro Arg Arg Gly Pro Pro Thr Tyr Arg Ala Asp Asp Glu Val
 165         170         175         180
Ile Cys Thr Ser Tyr Tyr Ser Arg Leu Val Pro Leu Glu His Gly Glu Ile His Thr Ser
 185         190         195         200
Leu Ile Asn Gly Arg Pro Ser Ala Asp Asp Leu Ser Pro Lys Leu Leu Glu Phe Thr Ser
 205         210         215         220
Ala Arg Tyr Ile Arg Leu Arg Leu Gln Arg Ile Arg Thr Leu Asn Ala Asp Leu Met Thr
 225         230         235         240
Leu Ser His Arg Glu Pro Lys Glu Leu Asp Pro Ile Val Thr Arg Arg Tyr Tyr Tyr Ser
 245         250         255         260
Ile Lys Asp Ile Ser Val Gly Gly Met Cys Ile Cys Tyr Gly His Ala Ser Ser Cys Pro
 265         270         275         280
Trp Asp Glu Thr Thr Lys Lys Leu Gln Cys Gln Cys Glu His Asn Thr Cys Gly Glu Ser
 285         290         295         300
Cys Asn Arg Cys Cys Pro Gly Tyr His Gln Gln Pro Trp Arg Pro Gly Thr Val Ser Ser
 305         310         315         320
Gly Asn Thr Cys Glu Ala Cys Asn Cys His Asn Lys Ala Lys Asp Cys Tyr Tyr Asp Glu
 325         330         335         340
Ser Val Ala Lys Gln Lys Lys Ser Leu Asn Thr Ala Gly Gln Phe Arg Gly Gly Gly Val
 345         350         355         360
Cys Ile Asn Cys Leu Gln Asn Thr Met Gly Ile Asn Cys Glu Thr Cys Ile Asp Gly Tyr
 365         370         375         380
Tyr Arg Pro His Lys Val Ser Pro Tyr Glu Asp Glu Pro Cys Arg Pro Cys Asn Cys Asp
 385         390         395         400
Pro Val Gly Ser Leu Ser Ser Val Cys Ile Lys Asp Asp Leu His Ser Asp Leu His Asn
 405         410         415         420

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Gly Lys Gln Pro	Gly Gln Cys Pro Cys	Lys Glu Gly Tyr Thr	Gly Glu Lys Cys Asp Arg
425		430	435 440
Cys Gln Leu Gly	Tyr Lys Asp Tyr Pro	Thr Cys Val Ser Cys	Gly Cys Asn Pro Val Gly
445		450	455 460
Ser Ala Ser Asp	Glu Pro Cys Thr Gly	Pro Cys Val Cys Lys	Glu Asn Val Glu Gly Lys
465		470	475 480
Ala Cys Asp Arg	Cys Lys Pro Gly Phe	Tyr Asn Leu Lys Glu	Lys Asn Pro Arg Gly Cys
485		490	495 500
Ser Glu Cys Phe	Cys Phe Gly Val Ser	Asp Val Cys Ser Ser	Leu Ser Trp Pro Val Gly
505		510	515 520
Gln Val Asn Ser	Met Ser Gly Trp Leu	Val Thr Asp Leu Ile	Ser Pro Arg Lys Ile Pro
525		530	535 540
Ser Gln Gln Asp	Ala Leu Gly Gly Arg	His Gln Val Ser Ile	Asn Asn Thr Ala Val Met
545		550	555 560
Gln Arg Leu Ala	Pro Lys Tyr Tyr Trp	Ala Ala Pro Glu Ala	Tyr Leu Gly Asn Lys Leu
565		570	575 580
Thr Ala Phe Gly	Gly Phe Leu Lys Tyr	Thr Val Ser Tyr Asp	Ile Pro Val Glu Thr Val
585		590	595 600
Asp Ser Asn Leu	Met Ser His Ala Asp	Val Ile Ile Lys Gly	Asn Gly Leu Thr Leu Ser
605		610	615 620
Thr Gln Ala Glu	Gly Leu Ser Leu Gln	Pro Tyr Glu Glu Tyr	Leu Asn Val Val Arg Leu
625		630	635 640
Val Pro Glu Asn	Phe Gln Asp Phe His	Ser Lys Arg Gln Ile	Asp Arg Asp Gln Leu Met
645		650	655 660
Thr Val Leu Ala	Asn Val Thr His Leu	Leu Ile Arg Ala Thr	Tyr Asn Ser Ala Lys Met
665		670	675 680
Ala Leu Tyr Arg	Leu Glu Ser Val Ser	Leu Asp Ile Ala Ser	Ser Asn Ala Ile Asp Leu
685		690	695 700
Val Val Ala Ala	Asp Val Glu His Cys	Glu Cys Pro Gln Gly	Tyr Thr Gly Thr Ser Cys
705		710	715 720
Glu Ser Cys Leu	Ser Gly Tyr Tyr Arg	Val Asp Gly Ile Leu	Phe Gly Gly Ile Cys Gln
725		730	735 740
Pro Cys Glu Cys	His Gly His Ala Ala	Glu Cys Asn Val His	Gly Val Cys Ile Ala Cys
745		750	755 760
Ala His Asn Thr	Thr Gly Val His Cys	Glu Gln Cys Leu Pro	Gly Phe Tyr Gly Glu Pro
765		770	775 780
Ser Arg Gly Thr	Pro Gly Asp Cys Gln	Pro Cys Ala Cys Pro	Leu Thr Ile Ala Ser Asn
785		790	795 800
Asn Phe Ser Pro	Thr Cys His Leu Asn	Asp Gly Asp Glu Val	Val Cys Asp Trp Cys Ala
805		810	815 820
Pro Gly Tyr Ser	Gly Ala Trp Cys Glu	Arg Cys Ala Asp Gly	Tyr Tyr Gly Asn Pro Thr
825		830	835 840
Val Pro Gly Glu	Ser Cys Val Pro Cys	Asp Cys Ser Gly Asn	Val Asp Pro Ser Glu Ala
845		850	855 860
Gly His Cys Asp	Ser Val Thr Gly Glu	Cys Leu Lys Cys Leu	Gly Asn Thr Asp Gly Ala
865		870	875 880
His Cys Glu Arg	Cys Ala Asp Gly Phe	Tyr Gly Asp Ala Val	Thr Ala Lys Asn Cys Arg
885		890	895 900
Ala Cys Glu Cys	His Val Lys Gly Ser	His Ser Ala Val Cys	His Leu Glu Thr Gly Leu
905		910	915 920
Cys Asp Cys Lys	Pro Asn Val Thr Gly	Gln Gln Cys Asp Gln	Cys Leu His Gly Tyr Tyr
925		930	935 940
Gly Leu Asp Ser	Gly His Gly Cys Arg	Pro Cys Asn Cys Ser	Val Ala Gly Ser Val Ser
945		950	955 960

Asp Gly Cys Thr Asp Glu Gly Gln Cys His Cys Val Pro Gly Val Ala Gly Lys Arg Cys
 965 970 975 980
 Asp Arg Cys Ala His Gly Phe Tyr Ala Tyr Gln Asp Gly Ser Cys Thr Pro Cys Asp Cys
 985 990 995 1000
 Pro His Thr Gln Asn Thr Cys Asp Pro Glu Thr Gly Glu Cys Val Cys Pro Pro His Thr
 1005 1010 1015 1020
 Gln Gly Gly Lys Cys Glu Glu Cys Glu Asp Gly His Trp Gly Tyr Asp Ala Glu Val Gly
 1025 1030 1035 1040
 Cys Gln Ala Cys Asn Cys Ser Leu Val Gly Ser Thr His His Arg Cys Asp Val Val Thr
 1045 1050 1055 1060
 Gly His Cys Gln Cys Lys Ser Lys Phe Gly Gly Arg Ala Cys Asp Gln Cys Ser Leu Gly
 1065 1070 1075 1080
 Tyr Arg Asp Phe Pro Asp Cys Val Pro Cys Asp Cys Asp Leu Arg Gly Thr Ser Gly Asp
 1085 1090 1095 1100
 Ala Cys Asn Leu Glu Gln Gly Leu Cys Gly Cys Val Glu Glu Thr Gly Ala Cys Pro Cys
 1105 1110 1115 1120
 Lys Glu Asn Val Phe Gly Pro Gln Cys Asn Glu Cys Arg Glu Gly Thr Phe Ala Leu Arg
 1125 1130 1135 1140
 Ala Asp Asn Pro Leu Gly Cys Ser Pro Cys Phe Cys Ser Gly Leu Ser His Leu Cys Ser
 1145 1150 1155 1160
 Glu Leu Glu Asp Tyr Val Arg Thr Pro Val Thr Leu Gly Ser Asp Gln Pro Leu Leu Arg
 1165 1170 1175 1180
 Val Val Ser Gln Ser Asn Leu Arg Gly Thr Thr Glu Gly Val Tyr Tyr Gln Ala Pro Asp
 1185 1190 1195 1200
 Phe Leu Leu Asp Ala Ala Thr Val Arg Gln His Ile Arg Ala Glu Pro Phe Tyr Trp Arg
 1205 1210 1215 1220
 Leu Pro Gln Gln Phe Gln Gly Asp Gln Leu Met Ala Tyr Gly Gly Lys Leu Lys Tyr Ser
 1225 1230 1235 1240
 Val Ala Phe Tyr Ser Leu Asp Gly Val Gly Thr Ser Asn Phe Glu Pro Gln Val Leu Ile
 1245 1250 1255 1260
 Lys Gly Gly Arg Ile Arg Lys Gln Val Ile Tyr Met Asp Ala Pro Ala Pro Glu Asn Gly
 1265 1270 1275 1280
 Val Arg Gln Glu Gln Glu Val Ala Met Arg Glu Asn Phe Trp Lys Tyr Phe Asn Ser Val
 1285 1290 1295 1300
 Ser Glu Lys Pro Val Thr Arg Glu Asp Phe Met Ser Val Leu Ser Asp Ile Glu Tyr Ile
 1305 1310 1315 1320
 Leu Ile Lys Ala Ser Tyr Gly Gln Gly Leu Gln Gln Ser Arg Ile Ser Asp Ile Ser Val
 1325 1330 1335 1340
 Glu Val Gly Arg Lys Ala Glu Lys Leu His Pro Glu Glu Glu Val Ala Ser Leu Leu Glu
 1345 1350 1355 1360
 Asn Cys Val Cys Pro Pro Gly Thr Val Gly Phe Ser Cys Gln Asp Cys Ala Pro Gly Tyr
 1365 1370 1375 1380
 His Arg Gly Lys Leu Pro Ala Gly Ser Asp Arg Gly Pro Arg Pro Leu Val Ala Pro Cys
 1385 1390 1395 1400
 Val Pro Cys Ser Cys Asn Asn His Ser Asp Thr Cys Asp Pro Asn Thr Gly Lys Cys Leu
 1405 1410 1415 1420
 Asn Cys Gly Asp Asn Thr Ala Gly Asp His Cys Asp Val Cys Thr Ser Gly Tyr Tyr Gly
 1425 1430 1435 1440
 Lys Val Thr Gly Ser Ala Ser Asp Cys Ala Leu Cys Ala Cys Pro His Ser Pro Pro Ala
 1445 1450 1455 1460
 Ser Phe Ser Pro Thr Cys Val Leu Glu Gly Asp His Asp Phe Arg Cys Asp Ala Cys Leu
 1465 1470 1475 1480
 Leu Gly Tyr Glu Gly Lys His Cys Glu Arg Cys Ser Ser Ser Tyr Tyr Gly Asn Pro Gln
 1485 1490 1495 1500

Thr Pro Gly Gly Ser Cys Gln Lys Cys Asp Cys Asn Arg His Gly Ser Val His Gly Asp	1505	1510	1515	1520
Cys Asp Arg Thr Ser Gly Gln Cys Val Cys Arg Leu Gly Ala Ser Gly Leu Arg Cys Asp	1525	1530	1535	1540
Glu Cys Glu Pro Arg His Ile Leu Met Glu Thr Asp Cys Val Ser Cys Asp Asp Glu Cys	1545	1550	1555	1560
Val Gly Val Leu Leu Asn Asp Leu Asp Glu Ile Gly Asp Ala Val Leu Ser Leu Asn Leu	1565	1570	1575	1580
Thr Gly Ile Ile Pro Val Pro Tyr Gly Ile Leu Ser Asn Leu Glu Asn Thr Thr Lys Tyr	1585	1590	1595	1600
Leu Gln Glu Ser Leu Leu Lys Glu Asn Met Gln Lys Asp Leu Gly Lys Ile Lys Leu Glu	1605	1610	1615	1620
Gly Val Ala Glu Glu Thr Asp Asn Leu Gln Lys Lys Leu Thr Arg Met Leu Ala Ser Thr	1625	1630	1635	1640
Gln Lys Val Asn Arg Ala Thr Glu Arg Ile Phe Lys Glu Ser Gln Asp Leu Ala Val Ala	1645	1650	1655	1660
Ile Glu Arg Leu Gln Met Ser Ile Thr Glu Ile Met Glu Lys Thr Thr Leu Asn Gln Thr	1665	1670	1675	1680
Leu Asp Glu Asp Phe Leu Leu Pro Asn Ser Thr Leu Gln Asn Met Gln Gln Asn Gly Thr	1685	1690	1695	1700
Ser Leu Leu Glu Ile Met Gln Ile Arg Asp Phe Thr Gln Leu His Gln Asn Ala Thr Leu	1705	1710	1715	1720
Glu Leu Lys Ala Ala Glu Asp Leu Leu Ser Gln Ile Gln Glu Asn Tyr Gln Lys Pro Leu	1725	1730	1735	1740
Glu Glu Leu Glu Val Leu Lys Glu Ala Ala Ser His Val Leu Ser Lys His Asn Asn Glu	1745	1750	1755	1760
Leu Lys Ala Ala Glu Ala Leu Val Arg Glu Ala Glu Ala Lys Met Gln Glu Ser Asn His	1765	1770	1775	1780
Leu Leu Leu Met Val Asn Ala Asn Leu Arg Glu Phe Ser Asp Lys Lys Leu His Val Gln	1785	1790	1795	1800
Glu Glu Gln Asn Leu Thr Ser Glu Leu Ile Val Gln Gly Arg Gly Leu Ile Asp Ala Ala	1805	1810	1815	1820
Ala Ala Gln Thr Asp Ala Val Gln Asp Ala Leu Glu His Leu Glu Asp His Gln Asp Lys	1825	1830	1835	1840
Leu Leu Leu Trp Ser Ala Lys Ile Arg His His Ile Asp Asp Leu Val Met His Met Ser	1845	1850	1855	1860
Gln Arg Asn Ala Val Asp Leu Val Tyr Arg Ala Glu Asp His Ala Thr Glu Phe Gln Arg	1865	1870	1875	1880
Leu Ala Asp Val Leu Tyr Ser Gly Leu Glu Asn Ile Arg Asn Val Ser Leu Asn Ala Thr	1885	1890	1895	1900
Ser Ala Ala Tyr Val His Tyr Asn Ile Gln Ser Leu Ile Glu Glu Ser Glu Glu Leu Ala	1905	1910	1915	1920
Arg Asp Ala His Arg Thr Val Thr Glu Thr Ser Leu Leu Ser Glu Ser Leu Val Ser Asn	1925	1930	1935	1940
Gly Lys Ala Ala Val Gln Arg Ser Ser Arg Phe Leu Lys Glu Gly Asn Asn Leu Ser Arg	1945	1950	1955	1960
Lys Leu Pro Gly Ile Ala Leu Glu Leu Ser Glu Leu Arg Asn Lys Thr Asn Arg Phe Gln	1965	1970	1975	1980
Glu Asn Ala Val Glu Ile Thr Arg Gln Thr Asn Glu Ser Leu Leu Ile Leu Arg Ala Ile	1985	1990	1995	2000
Pro Glu Gly Ile Arg Asp Lys Gly Ala Lys Thr Lys Glu Leu Ala Thr Ser Ala Ser Gln	2005	2010	2015	2020
Ser Ala Val Ser Thr Leu Arg Asp Val Ala Gly Leu Ser Gln Glu Leu Leu Asn Thr Ser	2025	2030	2035	2040

Ala Ser Leu Ser Arg Val Asn Thr Thr Leu Arg Glu Thr His Gln Leu Leu Gln Asp Ser
 2045 2050 2055 2060
 Thr Met Ala Thr Leu Leu Ala Gly Arg Lys Val Lys Asp Val Glu Ile Gln Ala Asn Leu
 2065 2070 2075 2080
 Leu Phe Asp Arg Leu Lys Pro Leu Lys Met Leu Glu Glu Asn Leu Ser Arg Asn Leu Ser
 2085 2090 2095 2100
 Glu Ile Lys Leu Leu Ile Ser Gln Ala Arg Lys Gln Ala Ala Ser Ile Lys Val Ala Val
 2105 2110 2115 2120
 Ser Ala Asp Arg Asp Cys Ile Arg Ala Tyr Gln Pro Gln Ile Ser Ser Thr Asn Tyr Asn
 2125 2130 2135 2140
 Thr Leu Thr Leu Asn Val Lys Thr Gln Glu Pro Asp Asn Leu Leu Phe Tyr Leu Gly Ser
 2145 2150 2155 2160
 Ser Thr Ala Ser Asp Phe Leu Ala Val Glu Met Arg Arg Gly Arg Val Ala Phe Leu Trp
 2165 2170 2175 2180
 Asp Leu Gly Ser Gly Ser Thr Arg Leu Glu Phe Pro Asp Phe Pro Ile Asp Asp Asn Arg
 2185 2190 2195 2200
 Trp His Ser Ile His Val Ala Arg Phe Gly Asn Ile Gly Ser Leu Ser Val Lys Glu Met
 2205 2210 2215 2220
 Ser Ser Asn Gln Lys Ser Pro Thr Lys Thr Ser Lys Ser Pro Gly Thr Ala Asn Val Leu
 2225 2230 2235 2240
 Asp Val Asn Asn Ser Thr Leu Met Phe Val Gly Gly Leu Gly Gly Gln Ile Lys Lys Ser
 2245 2250 2255 2260
 Pro Ala Val Lys Val Thr His Phe Lys Gly Cys Leu Gly Glu Ala Phe Leu Asn Gly Lys
 2265 2270 2275 2280
 Ser Ile Gly Leu Trp Asn Tyr Ile Glu Arg Glu Gly Lys Cys Arg Gly Cys Phe Gly Ser
 2285 2290 2295 2300
 Ser Gln Asn Glu Asp Pro Ser Phe His Phe Asp Gly Ser Gly Tyr Ser Val Val Glu Lys
 2305 2310 2315 2320
 Ser Leu Pro Ala Thr Val Thr Gln Ile Ile Met Leu Phe Asn Thr Phe Ser Pro Asn Gly
 2325 2330 2335 2340
 Leu Leu Leu Tyr Leu Gly Ser Tyr Gly Thr Lys Asp Phe Leu Ser Ile Glu Leu Phe Arg
 2345 2350 2355 2360
 Gly Arg Val Lys Val Met Thr Asp Leu Gly Ser Gly Pro Ile Thr Leu Leu Thr Asp Arg
 2365 2370 2375 2380
 Arg Tyr Asn Asn Gly Thr Trp Tyr Lys Ile Ala Phe Gln Arg Asn Arg Lys Gln Gly Val
 2385 2390 2395 2400
 Leu Ala Val Ile Asp Ala Tyr Asn Thr Ser Asn Lys Glu Thr Lys Gln Gly Glu Thr Pro
 2405 2410 2415 2420
 Gly Ala Ser Ser Asp Leu Asn Arg Leu Asp Lys Asp Pro Ile Tyr Val Gly Gly Leu Pro
 2425 2430 2435 2440
 Arg Ser Arg Val Val Arg Arg Gly Val Thr Thr Lys Ser Phe Val Gly Cys Ile Lys Asn
 2445 2450 2455 2460
 Leu Glu Ile Ser Arg Ser Thr Phe Asp Leu Leu Arg Asn Ser Tyr Gly Val Arg Lys Gly
 2465 2470 2475 2480
 Cys Leu Leu Glu Pro Ile Arg Ser Val Ser Phe Leu Lys Gly Gly Tyr Ile Glu Leu Pro
 2485 2490 2495 2500
 Pro Lys Ser Leu Ser Pro Glu Ser Glu Trp Leu Val Thr Phe Ala Thr Thr Asn Ser Ser
 2505 2510 2515 2520
 Gly Ile Ile Leu Ala Ala Leu Gly Gly Asp Val Glu Lys Arg Gly Asp Arg Glu Glu Ala
 2525 2530 2535 2540
 His Val Pro Phe Phe Ser Val Met Leu Ile Gly Gly Asn Ile Glu Val His Val Asn Pro
 2545 2550 2555 2560
 Gly Asp Gly Thr Gly Leu Arg Lys Ala Leu Leu His Ala Pro Thr Gly Thr Cys Ser Asp
 2565 2570 2575 2580

Gly Gln Ala His Ser Ile Ser Leu Val Arg Asn Arg Arg Ile Ile Thr Val Gln Leu Asp	2585	2590	2595	2600
Glu Asn Asn Pro Val Glu Met Lys Leu Gly Thr Leu Val Glu Ser Arg Thr Ile Asn Val	2605	2610	2615	2620
Ser Asn Leu Tyr Val Gly Gly Ile Pro Glu Gly Glu Gly Thr Ser Leu Leu Thr Met Arg	2625	2630	2635	2640
Arg Ser Phe His Gly Cys Ile Lys Asn Leu Ile Phe Asn Leu Glu Leu Leu Asp Phe Asn	2645	2650	2655	2660
Ser Ala Val Gly His Glu Gln Val Asp Leu Asp Thr Cys Trp Leu Ser Glu Arg Pro Lys	2665	2670	2675	2680
Leu Ala Pro Asp Ala Glu Asp Ser Lys Leu Leu Arg Glu Pro Arg Ala Phe Pro Glu Gln	2685	2690	2695	2700
Cys Val Val Asp Ala Ala Leu Glu Tyr Val Pro Gly Ala His Gln Phe Gly Leu Thr Gln	2705	2710	2715	2720
Asn Ser His Phe Ile Leu Pro Phe Asn Gln Ser Ala Val Arg Lys Lys Leu Ser Val Glu	2725	2730	2735	2740
Leu Ser Ile Arg Thr Phe Ala Ser Ser Gly Leu Ile Tyr Tyr Met Ala His Gln Asn Gln	2745	2750	2755	2760
Ala Asp Tyr Ala Val Leu Gln Leu His Gly Gly Arg Leu His Phe Met Phe Asp Leu Gly	2765	2770	2775	2780
Lys Gly Arg Thr Lys Val Ser His Pro Ala Leu Leu Ser Asp Gly Lys Trp His Thr Val	2785	2790	2795	2800
Lys Thr Asp Tyr Val Lys Arg Lys Gly Phe Ile Thr Val Asp Gly Arg Glu Ser Pro Met	2805	2810	2815	2820
Val Thr Val Val Gly Asp Gly Thr Met Leu Asp Val Glu Gly Leu Phe Tyr Leu Gly Gly	2825	2830	2835	2840
Leu Pro Ser Gln Tyr Gln Ala Arg Lys Ile Gly Asn Ile Thr His Ser Ile Pro Ala Cys	2845	2850	2855	2860
Ile Gly Asp Val Thr Val Asn Ser Lys Gln Leu Asp Lys Asp Ser Pro Val Ser Ala Phe	2865	2870	2875	2880
Thr Val Asn Arg Cys Tyr Ala Val Ala Gln Glu Gly Thr Tyr Phe Asp Gly Ser Gly Tyr	2885	2890	2895	2900
Ala Ala Leu Val Lys Glu Gly Tyr Lys Val Gln Ser Asp Val Asn Ile Thr Leu Glu Phe	2905	2910	2915	2920
Arg Thr Ser Ser Gln Asn Gly Val Leu Leu Gly Ile Ser Thr Ala Lys Val Asp Ala Ile	2925	2930	2935	2940
Gly Leu Glu Leu Val Asp Gly Lys Val Leu Phe His Val Asn Asn Gly Ala Gly Arg Ile	2945	2950	2955	2960
Thr Pro Ala Tyr Glu Pro Lys Thr Ala Thr Val Leu Cys Asp Gly Lys Trp His Thr Leu	2965	2970	2975	2980
Gln Ala Asn Lys Ser Lys His Arg Ile Thr Leu Ile Val Asp Gly Asn Ala Val Gly Ala	2985	2990	2995	3000
Glu Ser Pro His Thr Gln Ser Thr Ser Val Asp Thr Asn Asn Pro Ile Tyr Val Gly Gly	3005	3010	3015	3020
Tyr Pro Ala Gly Val Lys Gln Lys Cys Leu Arg Ser Gln Thr Ser Phe Arg Gly Cys Leu	3025	3030	3035	3040
Arg Lys Leu Ala Leu Ile Lys Ser Pro Gln Val Gln Ser Phe Asp Phe Ser Arg Ala Phe	3045	3050	3055	3060
Glu Leu His Gly Val Phe Leu His Ser Cys Pro Gly Thr Glu Ser	3065	3070	3075	

INFORMATION FOR SEQ ID NO: 6:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 1786 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P07942;

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 6:

Met	Gly	Leu	Leu	Gln	Leu	Leu	Ala	Phe	Ser	Phe	Leu	Ala	Leu	Cys	Arg	Ala	Arg	Val	Arg	1	5	10	15	20
Ala	Gln	Glu	Pro	Glu	Phe	Ser	Tyr	Gly	Cys	Ala	Glu	Gly	Ser	Cys	Tyr	Pro	Ala	Thr	Gly	25	30	35	40	
Asp	Leu	Leu	Ile	Gly	Arg	Ala	Gln	Lys	Leu	Ser	Val	Thr	Ser	Thr	Cys	Gly	Leu	His	Lys	45	50	55	60	
Pro	Glu	Pro	Tyr	Cys	Ile	Val	Ser	His	Leu	Gln	Glu	Asp	Lys	Lys	Cys	Phe	Ile	Cys	Asn	65	70	75	80	
Ser	Gln	Asp	Pro	Tyr	His	Glu	Thr	Leu	Asn	Pro	Asp	Ser	His	Leu	Ile	Glu	Asn	Val	Val	85	90	95	100	
Thr	Thr	Phe	Ala	Pro	Asn	Arg	Leu	Lys	Ile	Trp	Trp	Gln	Ser	Glu	Asn	Gly	Val	Glu	Asn	105	110	115	120	
Val	Thr	Ile	Gln	Leu	Asp	Leu	Glu	Ala	Glu	Phe	His	Phe	Thr	His	Leu	Ile	Met	Thr	Phe	125	130	135	140	
Lys	Thr	Phe	Arg	Pro	Ala	Ala	Met	Leu	Ile	Glu	Arg	Ser	Ser	Asp	Phe	Gly	Lys	Thr	Trp	145	150	155	160	
Gly	Val	Tyr	Arg	Tyr	Phe	Ala	Tyr	Asp	Cys	Glu	Ala	Ser	Phe	Pro	Gly	Ile	Ser	Thr	Gly	165	170	175	180	
Pro	Met	Lys	Lys	Val	Asp	Asp	Ile	Ile	Cys	Asp	Ser	Arg	Tyr	Ser	Asp	Ile	Glu	Pro	Ser	185	190	195	200	
Thr	Glu	Gly	Glu	Val	Ile	Phe	Arg	Ala	Leu	Asp	Pro	Ala	Phe	Lys	Ile	Glu	Asp	Pro	Tyr	205	210	215	220	
Ser	Pro	Arg	Ile	Gln	Asn	Leu	Leu	Lys	Ile	Thr	Asn	Leu	Arg	Ile	Lys	Phe	Val	Lys	Leu	225	230	235	240	
His	Thr	Leu	Gly	Asp	Asn	Leu	Leu	Asp	Ser	Arg	Met	Glu	Ile	Arg	Glu	Lys	Tyr	Tyr	Tyr	245	250	255	260	
Ala	Val	Tyr	Asp	Met	Val	Val	Arg	Gly	Asn	Cys	Phe	Cys	Tyr	Gly	His	Ala	Ser	Glu	Cys	265	270	275	280	
Ala	Pro	Val	Asp	Gly	Phe	Asn	Glu	Glu	Val	Glu	Gly	Met	Val	His	Gly	His	Cys	Met	Cys	285	290	295	300	
Arg	His	Asn	Thr	Lys	Gly	Leu	Asn	Cys	Glu	Leu	Cys	Met	Asp	Phe	Tyr	His	Asp	Leu	Pro	305	310	315	320	
Trp	Arg	Pro	Ala	Glu	Gly	Arg	Asn	Ser	Asn	Ala	Cys	Lys	Lys	Cys	Asn	Cys	Asn	Glu	His	325	330	335	340	
Ser	Ile	Ser	Cys	His	Phe	Asp	Met	Ala	Val	Tyr	Leu	Ala	Thr	Gly	Asn	Val	Ser	Gly	Gly	345	350	355	360	
Val	Cys	Asp	Asp	Cys	Gln	His	Asn	Thr	Met	Gly	Arg	Asn	Cys	Glu	Gln	Cys	Lys	Pro	Phe	365	370	375	380	
Tyr	Tyr	Gln	His	Pro	Glu	Arg	Asp	Ile	Arg	Asp	Pro	Asn	Phe	Cys	Glu	Arg	Cys	Thr	Cys	385	390	395	400	

Asp Pro Ala Gly	Ser Gln Asn Glu Gly	Ile Cys Asp Ser Tyr	Thr Asp Phe Ser Thr	Gly
405		410	415	420
Leu Ile Ala Gly	Gln Cys Arg Cys Lys	Leu Asn Val Glu Gly	Glu His Cys Asp Val	Cys
425		430	435	440
Lys Glu Gly Phe	Tyr Asp Leu Ser Ser	Glu Asp Pro Phe Gly	Cys Lys Ser Cys Ala	Cys
445		450	455	460
Asn Pro Leu Gly	Thr Ile Pro Gly Gly	Asn Pro Cys Asp Ser	Glu Thr Gly His Cys	Tyr
465		470	475	480
Cys Lys Arg Leu	Val Thr Gly Gln His	Cys Asp Gln Cys Leu	Pro Glu His Trp Gly	Leu
485		490	495	500
Ser Asn Asp Leu	Asp Gly Cys Arg Pro	Cys Asp Cys Asp Leu	Gly Gly Ala Leu Asn	Asn
505		510	515	520
Ser Cys Phe Ala	Glu Ser Gly Gln Cys	Ser Cys Arg Pro His	Met Ile Gly Arg Gln	Cys
525		530	535	540
Asn Glu Val Glu	Pro Gly Tyr Tyr Phe	Ala Thr Leu Asp His	Tyr Leu Tyr Glu Ala	Glu
545		550	555	560
Glu Ala Asn Leu	Gly Pro Gly Val Ser	Ile Val Glu Arg Gln	Tyr Ile Gln Asp Arg	Ile
565		570	575	580
Pro Ser Trp Thr	Gly Ala Gly Phe Val	Arg Val Pro Glu Gly	Ala Tyr Leu Glu Phe	Phe
585		590	595	600
Ile Asp Asn Ile	Pro Tyr Ser Met Glu	Tyr Asp Ile Leu Ile	Arg Tyr Glu Pro Gln	Leu
605		610	615	620
Pro Asp His Trp	Glu Lys Ala Val Ile	Thr Val Gln Arg Pro	Gly Arg Ile Pro Thr	Ser
625		630	635	640
Ser Arg Cys Gly	Asn Thr Ile Pro Asp	Asp Asp Asn Gln Val	Val Ser Leu Ser Pro	Gly
645		650	655	660
Ser Arg Tyr Val	Val Leu Pro Arg Pro	Val Cys Phe Glu Lys	Gly Thr Asn Tyr Thr	Val
665		670	675	680
Arg Leu Glu Leu	Pro Gln Tyr Thr Ser	Ser Asp Ser Asp Val	Glu Ser Pro Tyr Thr	Leu
685		690	695	700
Ile Asp Ser Leu	Val Leu Met Pro Tyr	Cys Lys Ser Leu Asp	Ile Phe Thr Val Gly	Gly
705		710	715	720
Ser Gly Asp Gly	Val Val Thr Asn Ser	Ala Trp Glu Thr Phe	Gln Arg Tyr Arg Cys	Leu
725		730	735	740
Glu Asn Ser Arg	Ser Val Val Lys Thr	Pro Met Thr Asp Val	Cys Arg Asn Ile Ile	Phe
745		750	755	760
Ser Ile Ser Ala	Leu Leu His Gln Thr	Gly Leu Ala Cys Glu	Cys Asp Pro Gln Gly	Ser
765		770	775	780
Leu Ser Ser Val	Cys Asp Pro Asn Gly	Gly Gln Cys Gln Cys	Arg Pro Asn Val Val	Gly
785		790	795	800
Arg Thr Cys Asn	Arg Cys Ala Pro Gly	Thr Phe Gly Phe Gly	Pro Ser Gly Cys Lys	Pro
805		810	815	820
Cys Glu Cys His	Leu Gln Gly Ser Val	Asn Ala Phe Cys Asn	Pro Val Thr Gly Gln	Cys
825		830	835	840
His Cys Phe Gln	Gly Val Tyr Ala Arg	Gln Cys Asp Arg Cys	Leu Pro Gly His Trp	Gly
845		850	855	860
Phe Pro Ser Cys	Gln Pro Cys Gln Cys	Asn Gly His Ala Asp	Asp Cys Asp Pro Val	Thr
865		870	875	880
Gly Glu Cys Leu	Asn Cys Gln Asp Tyr	Thr Met Gly His Asn	Cys Glu Arg Cys Leu	Ala
885		890	895	900
Gly Tyr Tyr Gly	Asp Pro Ile Ile Gly	Ser Gly Asp His Cys	Arg Pro Cys Pro Cys	Pro
905		910	915	920
Asp Gly Pro Asp	Ser Gly Arg Gln Phe	Ala Arg Ser Cys Tyr	Gln Asp Pro Val Thr	Leu
925		930	935	940

Gln Leu Ala Cys Val Cys Asp Pro Gly Tyr Ile Gly Ser Arg Cys Asp Asp Cys Ala Ser
 945 950 955 960
 Gly Tyr Phe Gly Asn Pro Ser Glu Val Gly Gly Ser Cys Gln Pro Cys Gln Cys His Asn
 965 970 975 980
 Asn Ile Asp Thr Thr Asp Pro Glu Ala Cys Asp Lys Glu Thr Gly Arg Cys Leu Lys Cys
 985 990 995 1000
 Leu Tyr His Thr Glu Gly Glu His Cys Gln Phe Cys Arg Phe Gly Tyr Tyr Gly Asp Ala
 1005 1010 1015 1020
 Leu Arg Gln Asp Cys Arg Lys Cys Val Cys Asn Tyr Leu Gly Thr Val Gln Glu His Cys
 1025 1030 1035 1040
 Asn Gly Ser Asp Cys Gln Cys Asp Lys Ala Thr Gly Gln Cys Leu Cys Leu Pro Asn Val
 1045 1050 1055 1060
 Ile Gly Gln Asn Cys Asp Arg Cys Ala Pro Asn Thr Trp Gln Leu Ala Ser Gly Thr Gly
 1065 1070 1075 1080
 Cys Asp Pro Cys Asn Cys Asn Ala Ala His Ser Phe Gly Pro Ser Cys Asn Glu Phe Thr
 1085 1090 1095 1100
 Gly Gln Cys Gln Cys Met Pro Gly Phe Gly Gly Arg Thr Cys Ser Glu Cys Gln Glu Leu
 1105 1110 1115 1120
 Phe Trp Gly Asp Pro Asp Val Glu Cys Arg Ala Cys Asp Cys Asp Pro Arg Gly Ile Glu
 1125 1130 1135 1140
 Thr Pro Gln Cys Asp Gln Ser Thr Gly Gln Cys Val Cys Val Glu Gly Val Glu Gly Pro
 1145 1150 1155 1160
 Arg Cys Asp Lys Cys Thr Arg Gly Tyr Ser Gly Val Phe Pro Asp Cys Thr Pro Cys His
 1165 1170 1175 1180
 Gln Cys Phe Ala Leu Trp Asp Val Ile Ile Ala Glu Leu Thr Asn Arg Thr His Arg Phe
 1185 1190 1195 1200
 Leu Glu Lys Ala Lys Ala Leu Lys Ile Ser Gly Val Ile Gly Pro Tyr Arg Glu Thr Val
 1205 1210 1215 1220
 Asp Ser Val Glu Arg Lys Val Ser Glu Ile Lys Asp Ile Leu Ala Gln Ser Pro Ala Ala
 1225 1230 1235 1240
 Glu Pro Leu Lys Asn Ile Gly Asn Leu Phe Glu Glu Ala Glu Lys Leu Ile Lys Asp Val
 1245 1250 1255 1260
 Thr Glu Met Met Ala Gln Val Glu Val Lys Leu Ser Asp Thr Thr Ser Gln Ser Asn Ser
 1265 1270 1275 1280
 Thr Ala Lys Glu Leu Asp Ser Leu Gln Thr Glu Ala Glu Ser Leu Asp Asn Thr Val Lys
 1285 1290 1295 1300
 Glu Leu Ala Glu Gln Leu Glu Phe Ile Lys Asn Ser Asp Ile Arg Gly Ala Leu Asp Ser
 1305 1310 1315 1320
 Ile Thr Lys Tyr Phe Gln Met Ser Leu Glu Ala Glu Glu Arg Val Asn Ala Ser Thr Thr
 1325 1330 1335 1340
 Glu Pro Asn Ser Thr Val Glu Gln Ser Ala Leu Met Arg Asp Arg Val Glu Asp Val Met
 1345 1350 1355 1360
 Met Glu Arg Glu Ser Gln Phe Lys Glu Lys Gln Glu Glu Gln Ala Arg Leu Leu Asp Glu
 1365 1370 1375 1380
 Leu Ala Gly Lys Leu Gln Ser Leu Asp Leu Ser Ala Ala Ala Glu Met Thr Cys Gly Thr
 1385 1390 1395 1400
 Pro Pro Gly Ala Ser Cys Ser Glu Thr Glu Cys Gly Gly Pro Asn Cys Arg Thr Asp Glu
 1405 1410 1415 1420
 Gly Glu Arg Lys Cys Gly Gly Pro Gly Cys Gly Gly Leu Val Thr Val Ala His Asn Ala
 1425 1430 1435 1440
 Trp Gln Lys Ala Met Asp Leu Asp Gln Asp Val Leu Ser Ala Leu Ala Glu Val Glu Gln
 1445 1450 1455 1460
 Leu Ser Lys Met Val Ser Glu Ala Lys Leu Arg Ala Asp Glu Ala Lys Gln Ser Ala Glu
 1465 1470 1475 1480

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Asp Ile Leu Leu Lys Thr Asn Ala Thr Lys Glu Lys Met Asp Lys Ser Asn Glu Glu Leu	1485	1490	1495	1500
Arg Asn Leu Ile Lys Gln Ile Arg Asn Phe Leu Thr Gln Asp Ser Ala Asp Leu Asp Ser	1505	1510	1515	1520
Ile Glu Ala Val Ala Asn Glu Val Leu Lys Met Glu Met Pro Ser Thr Pro Gln Gln Leu	1525	1530	1535	1540
Gln Asn Leu Thr Glu Asp Ile Arg Glu Arg Val Glu Ser Leu Ser Gln Val Glu Val Ile	1545	1550	1555	1560
Leu Gln His Ser Ala Ala Asp Ile Ala Arg Ala Glu Met Leu Leu Glu Glu Ala Lys Arg	1565	1570	1575	1580
Ala Ser Lys Ser Ala Thr Asp Val Lys Val Thr Ala Asp Met Val Lys Glu Ala Leu Glu	1585	1590	1595	1600
Glu Ala Glu Lys Ala Gln Val Ala Ala Glu Lys Ala Ile Lys Gln Ala Asp Glu Asp Ile	1605	1610	1615	1620
Gln Gly Thr Gln Asn Leu Leu Thr Ser Ile Glu Ser Glu Thr Ala Ala Ser Glu Glu Thr	1625	1630	1635	1640
Leu Phe Asn Ala Ser Gln Arg Ile Ser Glu Leu Glu Arg Asn Val Glu Glu Leu Lys Arg	1645	1650	1655	1660
Lys Ala Ala Gln Asn Ser Gly Glu Ala Glu Tyr Ile Glu Lys Val Val Tyr Thr Val Lys	1665	1670	1675	1680
Gln Ser Ala Glu Asp Val Lys Lys Thr Leu Asp Gly Glu Leu Asp Glu Lys Tyr Lys Lys	1685	1690	1695	1700
Val Glu Asn Leu Ile Ala Lys Lys Thr Glu Glu Ser Ala Asp Ala Arg Arg Lys Ala Glu	1705	1710	1715	1720
Met Leu Gln Asn Glu Ala Lys Thr Leu Leu Ala Gln Ala Asn Ser Lys Leu Gln Leu Leu	1725	1730	1735	1740
Lys Asp Leu Glu Arg Lys Tyr Glu Asp Asn Gln Arg Tyr Leu Glu Asp Lys Ala Gln Glu	1745	1750	1755	1760
Leu Ala Arg Leu Glu Gly Glu Val Arg Ser Leu Leu Lys Asp Ile Ser Gln Lys Val Ala	1765	1770	1775	1780
Val Tyr Ser Thr Cys Leu	1785			

INFORMATION FOR SEQ ID NO: 7:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 1786 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P02469;

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 7:

Met	Gly	Leu	Leu	Gln	Val	Phe	Ala	Phe	Gly	Val	Leu	Ala	Leu	Trp	Gly	Thr	Arg	Val	Cys	1	5	10	15	20
Ala	Gln	Glu	Pro	Glu	Phe	Ser	Tyr	Gly	Cys	Ala	Glu	Gly	Ser	Cys	Tyr	Pro	Ala	Thr	Gly	25	30	35	40	
Asp	Leu	Leu	Ile	Gly	Arg	Ala	Gln	Lys	Leu	Ser	Val	Thr	Ser	Thr	Cys	Gly	Leu	His	Lys	45	50	55	60	
Pro	Glu	Pro	Tyr	Cys	Ile	Val	Ser	His	Leu	Gln	Glu	Asp	Lys	Lys	Cys	Phe	Ile	Cys	Asp	65	70	75	80	
Ser	Arg	Asp	Pro	Tyr	His	Glu	Thr	Leu	Asn	Pro	Asp	Ser	His	Leu	Ile	Glu	Asn	Val	Val	85	90	95	100	
Thr	Thr	Phe	Ala	Pro	Asn	Arg	Leu	Lys	Ile	Trp	Trp	Gln	Ser	Glu	Asn	Gly	Val	Glu	Asn	105	110	115	120	
Val	Thr	Ile	Gln	Leu	Asp	Leu	Glu	Ala	Glu	Phe	His	Phe	Thr	His	Leu	Ile	Met	Thr	Phe	125	130	135	140	
Lys	Thr	Phe	Arg	Pro	Ala	Ala	Met	Leu	Ile	Glu	Arg	Ser	Ser	Asp	Phe	Gly	Lys	Thr	Trp	145	150	155	160	
Gly	Val	Tyr	Arg	Tyr	Phe	Ala	Tyr	Asp	Cys	Glu	Ser	Ser	Phe	Pro	Gly	Ile	Ser	Thr	Gly	165	170	175	180	
Pro	Met	Lys	Lys	Val	Asp	Asp	Ile	Ile	Cys	Asp	Ser	Arg	Tyr	Ser	Asp	Ile	Glu	Pro	Ser	185	190	195	200	
Thr	Glu	Gly	Glu	Val	Ile	Phe	Arg	Ala	Leu	Asp	Pro	Ala	Phe	Lys	Ile	Glu	Asp	Pro	Tyr	205	210	215	220	
Ser	Pro	Arg	Ile	Gln	Asn	Leu	Leu	Lys	Ile	Thr	Asn	Leu	Arg	Ile	Lys	Phe	Val	Lys	Leu	225	230	235	240	
His	Thr	Leu	Gly	Asp	Asn	Leu	Leu	Asp	Ser	Arg	Met	Glu	Ile	Arg	Glu	Lys	Tyr	Tyr	Tyr	245	250	255	260	
Ala	Val	Tyr	Asp	Met	Val	Val	Arg	Gly	Asn	Cys	Phe	Cys	Tyr	Gly	His	Ala	Ser	Glu	Cys	265	270	275	280	
Ala	Pro	Val	Asp	Gly	Val	Asn	Glu	Glu	Val	Glu	Gly	Met	Val	His	Gly	His	Cys	Met	Cys	285	290	295	300	
Arg	His	Asn	Thr	Lys	Gly	Leu	Asn	Cys	Glu	Leu	Cys	Met	Asp	Phe	Tyr	His	Asp	Leu	Pro	305	310	315	320	
Trp	Arg	Pro	Ala	Glu	Gly	Arg	Asn	Ser	Asn	Ala	Cys	Lys	Lys	Cys	Asn	Cys	Asn	Glu	His	325	330	335	340	
Ser	Ser	Ser	Cys	His	Phe	Asp	Met	Ala	Val	Phe	Leu	Ala	Thr	Gly	Asn	Val	Ser	Gly	Gly	345	350	355	360	
Val	Cys	Asp	Asn	Cys	Gln	His	Asn	Thr	Met	Gly	Arg	Asn	Cys	Glu	Gln	Cys	Lys	Pro	Phe	365	370	375	380	
Tyr	Phe	Gln	His	Pro	Glu	Arg	Asp	Ile	Arg	Asp	Pro	Asn	Leu	Cys	Glu	Pro	Cys	Thr	Cys	385	390	395	400	

Asp Pro Ala Gly	Ser Glu Asn Gly Gly	Ile Cys Asp Gly Tyr	Thr Asp Phe Ser Val Gly
405		410	415 420
Leu Ile Ala Gly	Gln Cys Arg Cys Lys	Leu His Val Glu Gly	Glu Arg Cys Asp Val Cys
425		430	435 440
Lys Glu Gly Phe	Tyr Asp Leu Ser Ala	Glu Asp Pro Tyr Gly	Cys Lys Ser Cys Ala Cys
445		450	455 460
Asn Pro Leu Gly	Thr Ile Pro Gly Gly	Asn Pro Cys Asp Ser	Glu Thr Gly Tyr Cys Tyr
465		470	475 480
Cys Lys Arg Leu	Val Thr Gly Gln Arg	Cys Asp Gln Cys Leu	Pro Gln His Trp Gly Leu
485		490	495 500
Ser Asn Asp Leu	Asp Gly Cys Arg Pro	Cys Asp Cys Asp Leu	Gly Gly Ala Leu Asn Asn
505		510	515 520
Ser Cys Ser Glu	Asp Ser Gly Gln Cys	Ser Cys Leu Pro His	Met Ile Gly Arg Gln Cys
525		530	535 540
Asn Glu Val Glu	Ser Gly Tyr Tyr Phe	Thr Thr Leu Asp His	Tyr Ile Tyr Glu Ala Glu
545		550	555 560
Glu Ala Asn Leu	Gly Pro Gly Val Val	Val Val Glu Arg Gln	Tyr Ile Gln Asp Arg Ile
565		570	575 580
Pro Ser Trp Thr	Gly Pro Gly Phe Val	Arg Val Pro Glu Gly	Ala Tyr Leu Glu Phe Phe
585		590	595 600
Ile Asp Asn Ile	Pro Tyr Ser Met Glu	Tyr Glu Ile Leu Ile	Arg Tyr Glu Pro Gln Leu
605		610	615 620
Pro Asp His Trp	Glu Lys Ala Val Ile	Thr Val Gln Arg Pro	Gly Lys Ile Pro Ala Ser
625		630	635 640
Ser Arg Cys Gly	Asn Thr Val Pro Asp	Asp Asp Asn Gln Val	Val Ser Leu Ser Pro Gly
645		650	655 660
Ser Arg Tyr Val	Val Leu Pro Arg Pro	Val Cys Phe Glu Lys	Gly Met Asn Tyr Thr Val
665		670	675 680
Arg Leu Glu Leu	Pro Gln Tyr Thr Ala	Ser Gly Ser Asp Val	Glu Ser Pro Tyr Thr Phe
685		690	695 700
Ile Asp Ser Leu	Val Leu Met Pro Tyr	Cys Lys Ser Leu Asp	Ile Phe Thr Val Gly Gly
705		710	715 720
Ser Gly Asp Gly	Glu Val Thr Asn Ser	Ala Trp Glu Thr Phe	Gln Arg Tyr Arg Cys Leu
725		730	735 740
Glu Asn Ser Arg	Ser Val Val Lys Thr	Pro Met Thr Asp Val	Cys Arg Asn Ile Ile Phe
745		750	755 760
Ser Ile Ser Ala	Leu Ile His Gln Thr	Gly Leu Ala Cys Glu	Cys Asp Pro Gln Gly Ser
765		770	775 780
Leu Ser Ser Val	Cys Asp Pro Asn Gly	Gly Gln Cys Gln Cys	Arg Pro Asn Val Val Gly
785		790	795 800
Arg Thr Cys Asn	Arg Cys Ala Pro Gly	Thr Phe Gly Phe Gly	Pro Asn Gly Cys Lys Pro
805		810	815 820
Cys Asp Cys His	Leu Gln Gly Ser Ala	Ser Ala Phe Cys Asp	Ala Ile Thr Gly Gln Cys
825		830	835 840
His Cys Phe Gln	Gly Ile Tyr Ala Arg	Gln Cys Asp Arg Cys	Leu Pro Gly Tyr Trp Gly
845		850	855 860
Phe Pro Ser Cys	Gln Pro Cys Gln Cys	Asn Gly His Ala Leu	Asp Cys Asp Thr Val Thr
865		870	875 880
Gly Glu Cys Leu	Ser Cys Gln Asp Tyr	Thr Thr Gly His Asn	Cys Glu Arg Cys Leu Ala
885		890	895 900
Gly Tyr Tyr Gly	Asp Pro Ile Ile Gly	Ser Gly Asp His Cys	Arg Pro Cys Pro Cys Pro
905		910	915 920
Asp Gly Pro Asp	Ser Gly Arg Gln Phe	Ala Arg Ser Cys Tyr	Gln Asp Pro Val Thr Leu
925		930	935 940

Gln Leu Ala Cys Val Cys Asp Pro Gly Tyr Ile Gly Ser Arg Cys Asp Asp Cys Ala Ser	945	950	955	960
Gly Phe Phe Gly Asn Pro Ser Asp Phe Gly Gly Ser Cys Gln Pro Cys Gln Cys His His	965	970	975	980
Asn Ile Asp Thr Thr Asp Pro Glu Ala Cys Asp Lys Asp Thr Gly Arg Cys Leu Lys Cys	985	990	995	1000
Leu Tyr His Thr Glu Gly Asp His Cys Gln Leu Cys Gln Tyr Gly Tyr Tyr Gly Asp Ala	1005	1010	1015	1020
Leu Arg Gln Asp Cys Arg Lys Cys Val Cys Asn Tyr Leu Gly Thr Val Lys Glu His Cys	1025	1030	1035	1040
Asn Gly Ser Asp Cys His Cys Asp Lys Ala Thr Gly Gln Cys Ser Cys Leu Pro Asn Val	1045	1050	1055	1060
Ile Gly Gln Asn Cys Asp Arg Cys Ala Pro Asn Thr Trp Gln Leu Ala Ser Gly Thr Gly	1065	1070	1075	1080
Cys Gly Pro Cys Asn Cys Asn Ala Ala His Ser Phe Gly Pro Ser Cys Asn Glu Phe Thr	1085	1090	1095	1100
Gly Gln Cys Gln Cys Met Pro Gly Phe Gly Gly Arg Thr Cys Ser Glu Cys Gln Glu Leu	1105	1110	1115	1120
Phe Trp Gly Asp Pro Asp Val Glu Cys Arg Ala Cys Asp Cys Asp Pro Arg Gly Ile Glu	1125	1130	1135	1140
Thr Pro Gln Cys Asp Gln Ser Thr Gly Gln Cys Val Cys Val Glu Gly Val Glu Gly Pro	1145	1150	1155	1160
Arg Cys Asp Lys Cys Thr Arg Gly Tyr Ser Gly Val Phe Pro Asp Cys Thr Pro Cys His	1165	1170	1175	1180
Gln Cys Phe Ala Leu Trp Asp Ala Ile Ile Gly Glu Leu Thr Asn Arg Thr His Lys Phe	1185	1190	1195	1200
Leu Glu Lys Ala Lys Ala Leu Lys Ile Ser Gly Val Ile Gly Pro Tyr Arg Glu Thr Val	1205	1210	1215	1220
Asp Ser Val Glu Lys Lys Val Asn Glu Ile Lys Asp Ile Leu Ala Gln Ser Pro Ala Ala	1225	1230	1235	1240
Glu Pro Leu Lys Asn Ile Gly Ile Leu Phe Glu Glu Ala Glu Lys Leu Thr Lys Asp Val	1245	1250	1255	1260
Thr Glu Lys Met Ala Gln Val Glu Val Lys Leu Thr Asp Thr Ala Ser Gln Ser Asn Ser	1265	1270	1275	1280
Thr Ala Gly Glu Leu Gly Ala Leu Gln Ala Glu Ala Glu Ser Leu Asp Lys Thr Val Lys	1285	1290	1295	1300
Glu Leu Ala Glu Gln Leu Glu Phe Ile Lys Asn Ser Asp Ile Gln Gly Ala Leu Asp Ser	1305	1310	1315	1320
Ile Thr Lys Tyr Phe Gln Met Ser Leu Glu Ala Glu Lys Arg Val Asn Ala Ser Thr Thr	1325	1330	1335	1340
Asp Pro Asn Ser Thr Val Glu Gln Ser Ala Leu Thr Arg Asp Arg Val Glu Asp Leu Met	1345	1350	1355	1360
Leu Glu Arg Glu Ser Pro Phe Lys Glu Gln Gln Glu Gln Ala Arg Leu Leu Asp Glu	1365	1370	1375	1380
Leu Ala Gly Lys Leu Gln Ser Leu Asp Leu Ser Ala Ala Ala Gln Met Thr Cys Gly Thr	1385	1390	1395	1400
Pro Pro Gly Ala Asp Cys Ser Glu Ser Glu Cys Gly Gly Pro Asn Cys Arg Thr Asp Glu	1405	1410	1415	1420
Gly Glu Lys Lys Cys Gly Gly Pro Gly Cys Gly Gly Leu Val Thr Val Ala His Ser Ala	1425	1430	1435	1440
Trp Gln Lys Ala Met Asp Phe Asp Arg Asp Val Leu Ser Ala Leu Ala Glu Val Glu Gln	1445	1450	1455	1460
Leu Ser Lys Met Val Ser Glu Ala Lys Val Arg Ala Asp Glu Ala Lys Gln Asn Ala Gln	1465	1470	1475	1480

Asp Val Leu Leu Lys Thr Asn Ala Thr Lys Glu Lys Val Asp Lys Ser Asn Glu Asp Leu	1485	1490	1495	1500
Arg Asn Leu Ile Lys Gln Ile Arg Asn Phe Leu Thr Glu Asp Ser Ala Asp Leu Asp Ser	1505	1510	1515	1520
Ile Glu Ala Val Ala Asn Glu Val Leu Lys Ser Gly Asn Ala Ser Thr Pro Gln Gln Leu	1525	1530	1535	1540
Gln Asn Leu Thr Glu Asp Ile Arg Glu Arg Val Glu Thr Leu Ser Gln Val Glu Val Ile	1545	1550	1555	1560
Leu Gln Gln Ser Ala Ala Asp Ile Ala Arg Ala Glu Leu Leu Leu Glu Glu Ala Lys Arg	1565	1570	1575	1580
Ala Ser Lys Ser Ala Thr Asp Val Lys Val Thr Ala Asp Met Val Lys Glu Ala Leu Glu	1585	1590	1595	1600
Glu Ala Glu Lys Ala Gln Val Ala Ala Glu Lys Ala Ile Lys Gln Ala Asp Glu Asp Ile	1605	1610	1615	1620
Gln Gly Thr Gln Asn Leu Leu Thr Ser Ile Glu Ser Glu Thr Ala Ala Ser Glu Glu Thr	1625	1630	1635	1640
Leu Thr Asn Ala Ser Gln Arg Ile Ser Lys Leu Glu Arg Asn Val Glu Glu Leu Lys Arg	1645	1650	1655	1660
Lys Ala Ala Gln Asn Ser Gly Glu Ala Glu Tyr Ile Glu Lys Val Val Tyr Ser Val Lys	1665	1670	1675	1680
Gln Asn Ala Asp Asp Val Lys Lys Thr Leu Asp Gly Glu Leu Asp Glu Lys Tyr Lys Lys	1685	1690	1695	1700
Val Glu Ser Leu Ile Ala Gln Lys Thr Glu Glu Ser Ala Asp Ala Arg Arg Lys Ala Glu	1705	1710	1715	1720
Leu Leu Gln Asn Glu Ala Lys Thr Leu Leu Ala Gln Ala Asn Ser Lys Leu Gln Leu Leu	1725	1730	1735	1740
Glu Asp Leu Glu Arg Lys Tyr Glu Asp Asn Gln Lys Tyr Leu Glu Asp Lys Ala Gln Glu	1745	1750	1755	1760
Leu Val Arg Leu Glu Gly Glu Val Arg Ser Leu Leu Lys Asp Ile Ser Glu Lys Val Ala	1765	1770	1775	1780
Val Tyr Ser Thr Cys Leu	1785			

INFORMATION FOR SEQ ID NO: 8:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 1801 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P15800;

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 8:

```

Met Glu Trp Ala Ser Gly Lys Pro Gly Arg Gly Arg Gln Gly Gln Pro Val Pro Trp Glu
 1          5          10          15          20
Leu Arg Leu Gly Leu Leu Leu Ser Val Leu Ala Ala Thr Leu Ala Gln Val Pro Ser Leu
          25          30          35          40
Asp Val Pro Gly Cys Ser Arg Gly Ser Cys Tyr Pro Ala Thr Gly Asp Leu Leu Val Gly
          45          50          55          60
Arg Ala Asp Arg Leu Thr Ala Ser Ser Thr Cys Gly Leu His Ser Pro Gln Pro Tyr Cys
          65          70          75          80
Ile Val Ser His Leu Gln Asp Glu Lys Lys Cys Phe Leu Cys Asp Ser Arg Arg Pro Phe
          85          90          95          100
Ser Ala Arg Asp Asn Pro Asn Ser His Arg Ile Gln Asn Val Val Thr Ser Phe Ala Pro
          105          110          115          120
Gln Arg Arg Thr Ala Trp Trp Gln Ser Glu Asn Gly Val Pro Met Val Thr Ile Gln Leu
          125          130          135          140
Asp Leu Glu Ala Glu Phe His Phe Thr His Leu Ile Met Thr Phe Lys Thr Phe Arg Pro
          145          150          155          160
Ala Ala Met Leu Val Glu Arg Ser Ala Asp Phe Gly Arg Thr Trp Arg Val Tyr Arg Tyr
          165          170          175          180
Phe Ser Tyr Asp Cys Gly Ala Asp Phe Pro Gly Ile Pro Leu Ala Pro Pro Arg Arg Trp
          185          190          195          200
Asp Asp Val Val Cys Glu Ser Arg Tyr Ser Glu Ile Glu Pro Ser Thr Glu Gly Glu Val
          205          210          215          220
Ile Tyr Arg Val Leu Asp Pro Ala Ile Pro Ile Pro Asp Pro Tyr Ser Ser Arg Ile Gln
          225          230          235          240
Asn Leu Leu Lys Ile Thr Asn Leu Arg Val Asn Leu Thr Arg Leu His Thr Leu Gly Asp
          245          250          255          260
Asn Leu Leu Asp Pro Arg Arg Glu Ile Arg Glu Lys Tyr Tyr Tyr Ala Leu Tyr Glu Leu
          265          270          275          280
Val Ile Arg Gly Asn Cys Phe Cys Tyr Gly His Ala Ser Gln Cys Ala Pro Ala Pro Gly
          285          290          295          300
Ala Pro Ala His Ala Glu Gly Met Val His Gly Ala Cys Ile Cys Lys His Asn Thr Arg
          305          310          315          320
Gly Leu Asn Cys Glu Gln Cys Gln Asp Phe Tyr Gln Asp Leu Pro Trp His Pro Ala Glu
          325          330          335          340
Asp Gly His Thr His Ala Cys Arg Lys Cys Glu Cys Asn Gly His Ser His Ser Cys His
          345          350          355          360
Phe Asp Met Ala Val Tyr Leu Ala Ser Gly Asn Val Ser Gly Gly Val Cys Asp Gly Cys
          365          370          375          380
Gln His Asn Thr Ala Gly Arg His Cys Glu Leu Cys Arg Pro Phe Phe Tyr Arg Asp Pro
          385          390          395          400

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Thr Lys Asp Met	Arg Asp Pro Ala Ala	Cys Arg Pro Cys Asp	Cys Asp Pro Met Gly	Ser
405		410	415	420
Gln Asp Gly Gly	Arg Cys Asp Ser His	Asp Asp Pro Val Leu	Gly Leu Val Ser Gly	Gln
425		430	435	440
Cys Arg Cys Lys	Glu His Val Val Gly	Thr Arg Cys Gln Gln	Cys Arg Asp Gly Phe	Phe
445		450	455	460
Gly Leu Ser Ala	Ser Asn Pro Arg Gly	Cys Gln Arg Cys Gln	Cys Asn Ser Arg Gly	Thr
465		470	475	480
Val Pro Gly Gly	Thr Pro Cys Asp Ser	Ser Ser Gly Thr Cys	Phe Cys Lys Arg Leu	Val
485		490	495	500
Thr Gly Asp Gly	Cys Asp Arg Cys Leu	Pro Gly His Trp Gly	Leu Ser His Asp Leu	Leu
505		510	515	520
Gly Cys Arg Pro	Cys Asp Cys Asp Val	Gly Gly Ala Leu Asp	Pro Gln Cys Asp Glu	Ala
525		530	535	540
Thr Gly Gln Cys	Pro Cys Arg Pro His	Met Ile Gly Arg Arg	Cys Glu Gln Val Gln	Pro
545		550	555	560
Gly Tyr Phe Arg	Pro Phe Leu Asp His	Leu Thr Trp Glu Ala	Glu Gly Ala His Gly	Gln
565		570	575	580
Val Leu Glu Val	Val Glu Arg Leu Val	Thr Asn Arg Glu Thr	Pro Ser Trp Thr Gly	Val
585		590	595	600
Gly Phe Val Arg	Leu Arg Glu Gly Gln	Glu Val Glu Phe Leu	Val Thr Ser Leu Pro	Arg
605		610	615	620
Ala Met Asp Tyr	Asp Leu Leu Leu Arg	Trp Glu Pro Gln Val	Pro Glu Gln Trp Ala	Glu
625		630	635	640
Leu Glu Leu Val	Val Gln Arg Pro Gly	Pro Val Ser Ala His	Ser Pro Cys Gly His	Val
645		650	655	660
Leu Pro Arg Asp	Asp Arg Ile Gln Gly	Met Leu His Pro Asn	Thr Arg Val Leu Val	Phe
665		670	675	680
Pro Arg Pro Val	Cys Leu Glu Pro Gly	Leu Ser Tyr Lys Leu	Lys Leu Lys Leu Thr	Gly
685		690	695	700
Thr Gly Gly Arg	Ala His Pro Glu Thr	Pro Tyr Ser Gly Ser	Gly Ile Leu Ile Asp	Ser
705		710	715	720
Leu Val Leu Gln	Pro His Val Leu Met	Leu Glu Met Phe Ser	Gly Gly Asp Ala Ala	Ala
725		730	735	740
Leu Glu Arg Arg	Thr Thr Phe Glu Arg	Tyr Arg Cys His Glu	Glu Gly Leu Met Pro	Ser
745		750	755	760
Lys Thr Pro Leu	Ser Glu Ala Cys Val	Pro Leu Leu Ile Ser	Ala Ser Ser Leu Val	Tyr
765		770	775	780
Asn Gly Ala Leu	Pro Cys Gln Cys Asp	Pro Gln Gly Ser Leu	Ser Ser Glu Cys Asn	Pro
785		790	795	800
His Gly Gly Gln	Cys Arg Cys Lys Pro	Gly Val Val Gly Arg	Arg Cys Asp Ala Cys	Ala
805		810	815	820
Thr Gly Tyr Tyr	Gly Phe Gly Pro Ala	Gly Cys Gln Ala Cys	Gln Cys Ser Pro Asp	Gly
825		830	835	840
Ala Leu Ser Ala	Leu Cys Glu Gly Thr	Ser Gly Gln Cys Leu	Cys Arg Thr Gly Ala	Phe
845		850	855	860
Gly Leu Arg Cys	Asp His Cys Gln Arg	Gly Gln Trp Gly Phe	Pro Asn Cys Arg Pro	Cys
865		870	875	880
Val Cys Asn Gly	Arg Ala Asp Glu Cys	Asp Ala His Thr Gly	Ala Cys Leu Gly Cys	Arg
885		890	895	900
Asp Tyr Thr Gly	Gly Glu His Cys Glu	Arg Cys Ile Ala Gly	Phe His Gly Asp Pro	Arg
905		910	915	920
Leu Pro Tyr Gly	Gly Gln Cys Arg Pro	Cys Pro Cys Pro Glu	Gly Pro Gly Ser Gln	Arg
925		930	935	940

His Phe Ala Thr Ser Cys His Arg Asp Gly Tyr Ser Gln Gln Ile Val Cys His Cys Arg
 945 950 955 960
 Ala Gly Tyr Thr Gly Leu Arg Cys Glu Ala Cys Ala Pro Gly His Phe Gly Asp Pro Ser
 965 970 975 980
 Lys Pro Gly Gly Arg Cys Gln Leu Cys Glu Cys Ser Gly Asn Ile Asp Pro Thr Asp Pro
 985 990 995 1000
 Gly Ala Cys Asp Pro His Thr Gly Gln Cys Leu Arg Cys Leu His His Thr Glu Gly Pro
 1005 1010 1015 1020
 His Cys Gly His Cys Lys Pro Gly Phe His Gly Gln Ala Ala Arg Gln Ser Cys His Arg
 1025 1030 1035 1040
 Cys Thr Cys Asn Leu Leu Gly Thr Asp Pro Gln Arg Cys Pro Ser Thr Asp Leu Cys His
 1045 1050 1055 1060
 Cys Asp Pro Ser Thr Gly Gln Cys Pro Cys Leu Pro His Val Gln Gly Leu Ser Cys Asp
 1065 1070 1075 1080
 Arg Cys Ala Pro Asn Phe Trp Asn Phe Thr Ser Gly Arg Gly Cys Gln Pro Cys Ala Cys
 1085 1090 1095 1100
 His Pro Ser Arg Ala Arg Gly Pro Thr Cys Asn Glu Phe Thr Gly Gln Cys His Cys His
 1105 1110 1115 1120
 Ala Gly Phe Gly Gly Arg Thr Cys Ser Glu Cys Gln Glu Leu His Trp Gly Asp Pro Gly
 1125 1130 1135 1140
 Leu Gln Cys Arg Ala Cys Asp Cys Asp Pro Arg Gly Ile Asp Lys Pro Gln Cys His Arg
 1145 1150 1155 1160
 Ser Thr Gly His Cys Ser Cys Arg Pro Gly Val Ser Gly Val Arg Cys Asp Gln Cys Ala
 1165 1170 1175 1180
 Arg Gly Phe Ser Gly Val Phe Pro Ala Cys His Pro Cys His Ala Cys Phe Gly Asp Trp
 1185 1190 1195 1200
 Asp Arg Val Val Gln Asp Leu Ala Ala Arg Thr Arg Arg Leu Glu Gln Trp Ala Gln Glu
 1205 1210 1215 1220
 Leu Gln Gln Thr Gly Val Leu Gly Ala Phe Glu Ser Ser Phe Leu Asn Leu Gln Gly Lys
 1225 1230 1235 1240
 Leu Gly Met Val Gln Ala Ile Val Ala Ala Arg Asn Thr Ser Ala Ala Ser Thr Ala Lys
 1245 1250 1255 1260
 Leu Val Glu Ala Thr Glu Gly Leu Arg His Glu Ile Gly Lys Thr Thr Glu Arg Leu Thr
 1265 1270 1275 1280
 Gln Leu Glu Ala Glu Leu Thr Asp Val Gln Asp Glu Asn Phe Asn Ala Asn His Ala Leu
 1285 1290 1295 1300
 Ser Gly Leu Glu Arg Asp Gly Leu Ala Leu Asn Leu Thr Leu Arg Gln Leu Asp Gln His
 1305 1310 1315 1320
 Leu Asp Ile Leu Lys His Ser Asn Phe Leu Gly Ala Tyr Asp Ser Ile Arg His Ala His
 1325 1330 1335 1340
 Ser Gln Ser Thr Glu Ala Glu Arg Arg Ala Asn Ala Ser Thr Phe Ala Ile Pro Ser Pro
 1345 1350 1355 1360
 Val Ser Asn Ser Ala Asp Thr Arg Arg Arg Ala Glu Val Leu Met Gly Ala Gln Arg Glu
 1365 1370 1375 1380
 Asn Phe Asn Arg Gln His Leu Ala Asn Gln Gln Ala Leu Gly Arg Leu Ser Thr His Thr
 1385 1390 1395 1400
 His Thr Leu Ser Leu Thr Gly Val Asn Glu Leu Val Cys Gly Ala Pro Gly Asp Ala Pro
 1405 1410 1415 1420
 Cys Ala Thr Ser Pro Cys Gly Gly Ala Gly Cys Arg Asp Glu Asp Gly Gln Pro Arg Cys
 1425 1430 1435 1440
 Gly Gly Leu Gly Cys Ser Gly Ala Ala Ala Thr Ala Asp Leu Ala Leu Gly Arg Ala Arg
 1445 1450 1455 1460
 His Thr Gln Ala Glu Leu Gln Arg Ala Leu Val Glu Gly Gly Ile Leu Ser Arg Val
 1465 1470 1475 1480

Ser Glu Thr Arg Arg Gln Ala Glu Glu Ala Gln Gln Arg Ala Gln Ala Ala Leu Asp Lys
 1485 1490 1495 1500
 Ala Asn Ala Ser Arg Gly Gln Val Glu Gln Ala Asn Gln Glu Leu Arg Glu Leu Ile Gln
 1505 1510 1515 1520
 Asn Val Lys Asp Phe Leu Ser Gln Glu Gly Ala Asp Pro Asp Ser Ile Glu Met Val Ala
 1525 1530 1535 1540
 Thr Arg Val Leu Asp Ile Ser Ile Pro Ala Ser Pro Glu Gln Ile Gln Arg Leu Ala Ser
 1545 1550 1555 1560
 Glu Ile Ala Glu Arg Val Arg Ser Leu Ala Asp Val Asp Thr Ile Leu Ala His Thr Met
 1565 1570 1575 1580
 Gly Asp Val Arg Arg Ala Glu Gln Leu Leu Gln Asp Ala Gln Arg Ala Arg Ser Arg Ala
 1585 1590 1595 1600
 Glu Gly Glu Arg Gln Lys Ala Glu Thr Val Gln Ala Ala Leu Glu Glu Ala Gln Arg Ala
 1605 1610 1615 1620
 Gln Gly Ala Ala Gln Gly Ala Ile Arg Gly Ala Val Val Asp Thr Lys Asn Thr Glu Gln
 1625 1630 1635 1640
 Thr Leu Gln Gln Val Gln Glu Arg Met Ala Gly Thr Glu Gln Ser Leu Asn Ser Ala Ser
 1645 1650 1655 1660
 Glu Arg Ala Arg Gln Leu His Ala Leu Leu Glu Ala Leu Lys Leu Lys Arg Ala Gly Asn
 1665 1670 1675 1680
 Ser Leu Ala Ala Ser Thr Ala Glu Glu Thr Ala Gly Ser Ala Gln Ser Arg Ala Arg Glu
 1685 1690 1695 1700
 Ala Glu Lys Gln Leu Arg Glu Gln Val Gly Asp Gln Tyr Gln Thr Val Arg Ala Leu Ala
 1705 1710 1715 1720
 Glu Arg Lys Ala Glu Gly Val Leu Ala Ala Gln Ala Arg Ala Glu Gln Leu Arg Asp Glu
 1725 1730 1735 1740
 Ala Arg Gly Leu Leu Gln Ala Ala Gln Asp Lys Leu Gln Arg Leu Gln Glu Leu Glu Gly
 1745 1750 1755 1760
 Thr Tyr Glu Glu Asn Glu Arg Glu Leu Glu Val Lys Ala Ala Gln Leu Asp Gly Leu Glu
 1765 1770 1775 1780
 Ala Arg Met Arg Ser Val Leu Gln Ala Ile Asn Leu Gln Val Gln Ile Tyr Asn Thr Cys
 1785 1790 1795 1800
 Gln

INFORMATION FOR SEQ ID NO: 9:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 1798 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P55268;

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 9:

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Met Glu Leu Thr Ser Arg Glu Arg Gly Arg Gly Gln Pro Leu Pro Trp Glu Leu Arg Leu
 1           5           10           15           20
Gly Leu Leu Leu Ser Val Leu Ala Ala Thr Leu Ala Gln Ala Pro Ala Pro Asp Val Pro
          25           30           35           40
Gly Cys Ser Arg Gly Ser Cys Tyr Pro Ala Thr Gly Asp Leu Leu Val Gly Arg Ala Asp
          45           50           55           60
Arg Leu Thr Ala Ser Ser Thr Cys Gly Leu Asn Gly Pro Gln Pro Tyr Cys Ile Val Ser
          65           70           75           80
His Leu Gln Asp Glu Lys Lys Cys Phe Leu Cys Asp Ser Arg Arg Pro Phe Ser Ala Arg
          85           90           95          100
Asp Asn Pro His Ser His Arg Ile Gln Asn Val Val Thr Ser Phe Ala Pro Gln Arg Arg
          105          110          115          120
Ala Ala Trp Trp Gln Ser Glu Asn Gly Ile Pro Ala Val Thr Ile Gln Leu Asp Leu Glu
          125          130          135          140
Ala Glu Phe His Phe Thr His Leu Ile Met Thr Phe Lys Thr Phe Arg Pro Ala Ala Met
          145          150          155          160
Leu Val Glu Arg Ser Ala Asp Phe Gly Arg Thr Trp His Val Tyr Arg Tyr Phe Ser Tyr
          165          170          175          180
Asp Cys Gly Ala Asp Phe Pro Gly Val Pro Leu Ala Pro Pro Arg His Trp Asp Asp Val
          185          190          195          200
Val Cys Glu Ser Arg Tyr Ser Glu Ile Glu Pro Ser Thr Glu Gly Glu Val Ile Tyr Arg
          205          210          215          220
Val Leu Asp Pro Ala Ile Pro Ile Pro Asp Pro Tyr Ser Ser Arg Ile Gln Asn Leu Leu
          225          230          235          240
Lys Ile Thr Asn Leu Arg Val Asn Leu Thr Arg Leu His Thr Leu Gly Asp Asn Leu Leu
          245          250          255          260
Asp Pro Arg Arg Glu Ile Arg Glu Lys Tyr Tyr Tyr Ala Leu Tyr Glu Leu Val Val Arg
          265          270          275          280
Gly Asn Cys Phe Cys Tyr Gly His Ala Ser Glu Cys Ala Pro Ala Pro Gly Ala Pro Ala
          285          290          295          300
His Ala Glu Gly Met Val His Gly Ala Cys Ile Cys Lys His Asn Thr Arg Gly Leu Asn
          305          310          315          320
Cys Glu Gln Cys Gln Asp Phe Tyr Arg Asp Leu Pro Trp Arg Pro Ala Glu Asp Gly His
          325          330          335          340
Ser His Ala Cys Arg Lys Cys Glu Cys His Gly His Thr His Ser Cys His Phe Asp Met
          345          350          355          360
Ala Val Tyr Leu Ala Ser Gly Asn Val Ser Gly Gly Val Cys Asp Gly Cys Gln His Asn
          365          370          375          380
Thr Ala Gly Arg His Cys Glu Leu Cys Arg Pro Phe Phe Tyr Arg Asp Pro Thr Lys Asp
          385          390          395          400

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Leu Arg Asp Pro Ala Val Cys Arg Ser	Cys Asp Cys Asp Pro Met Gly Ser Gln Asp Gly	405	410	415	420
Gly Arg Cys Asp Ser His Asp Asp Pro	Ala Leu Gly Leu Val Ser Gly Gln Cys Arg Cys	425	430	435	440
Lys Glu His Val Val Gly Thr Arg Cys	Gln Gln Cys Arg Asp Gly Phe Phe Gly Leu Ser	445	450	455	460
Ile Ser Asp Arg Leu Gly Cys Arg Arg	Cys Gln Cys Asn Ala Arg Gly Thr Val Pro Gly	465	470	475	480
Ser Thr Pro Cys Asp Pro Asn Ser Gly	Ser Cys Tyr Cys Lys Arg Leu Val Thr Gly Arg	485	490	495	500
Gly Cys Asp Arg Cys Leu Pro Gly His	Trp Gly Leu Ser His Asp Leu Leu Gly Cys Arg	505	510	515	520
Pro Cys Asp Cys Asp Val Gly Gly Ala	Leu Asp Pro Gln Cys Asp Glu Gly Thr Gly Gln	525	530	535	540
Cys His Cys Arg Gln His Met Val Gly	Arg Arg Cys Glu Gln Val Gln Pro Gly Tyr Phe	545	550	555	560
Arg Pro Phe Leu Asp His Leu Ile Trp	Glu Ala Glu Asp Thr Arg Gly Gln Val Leu Asp	565	570	575	580
Val Val Glu Arg Leu Val Thr Pro Gly	Glu Thr Pro Ser Trp Thr Gly Ser Gly Phe Val	585	590	595	600
Arg Leu Gln Glu Gly Gln Thr Leu Glu	Phe Leu Val Ala Ser Val Pro Lys Ala Met Asp	605	610	615	620
Tyr Asp Leu Leu Leu Arg Leu Glu Pro	Gln Val Pro Glu Gln Trp Ala Glu Leu Glu Leu	625	630	635	640
Ile Val Gln Arg Pro Gly Pro Val Pro	Ala His Ser Leu Cys Gly His Leu Val Pro Lys	645	650	655	660
Asp Asp Arg Ile Gln Gly Thr Leu Gln	Pro His Ala Arg Tyr Leu Ile Phe Pro Asn Pro	665	670	675	680
Val Cys Leu Glu Pro Gly Ile Ser Tyr	Lys Leu His Leu Lys Leu Val Arg Thr Gly Gly	685	690	695	700
Ser Ala Gln Pro Glu Thr Pro Tyr Ser	Gly Pro Gly Leu Leu Ile Asp Ser Leu Val Leu	705	710	715	720
Leu Pro Arg Val Leu Val Leu Glu Met	Phe Ser Gly Gly Asp Ala Ala Ala Leu Glu Arg	725	730	735	740
Gln Ala Thr Phe Glu Arg Tyr Gln Cys	His Glu Glu Gly Leu Val Pro Ser Lys Thr Ser	745	750	755	760
Pro Ser Glu Ala Cys Ala Pro Leu Leu	Ile Ser Leu Ser Thr Leu Ile Tyr Asn Gly Ala	765	770	775	780
Leu Pro Cys Gln Cys Asn Pro Gln Gly	Ser Leu Ser Ser Glu Cys Asn Pro His Gly Gly	785	790	795	800
Gln Cys Leu Cys Lys Pro Gly Val Val	Gly Arg Arg Cys Asp Leu Cys Ala Pro Gly Tyr	805	810	815	820
Tyr Gly Phe Gly Pro Thr Gly Cys Gln	Ala Cys Gln Cys Ser His Glu Gly Ala Leu Ser	825	830	835	840
Ser Leu Cys Glu Lys Thr Ser Gly Gln	Cys Leu Cys Arg Thr Gly Ala Phe Gly Leu Arg	845	850	855	860
Cys Asp Arg Cys Gln Arg Gly Gln Trp	Gly Phe Pro Ser Cys Arg Pro Cys Val Cys Asn	865	870	875	880
Gly His Ala Asp Glu Cys Asn Thr His	Thr Gly Ala Cys Leu Gly Cys Arg Asp His Thr	885	890	895	900
Gly Gly Glu His Cys Glu Arg Cys Ile	Ala Gly Phe His Arg Asp Pro Arg Leu Pro Tyr	905	910	915	920
Gly Gly Gln Cys Arg Pro Cys Pro Cys	Pro Glu Gly Pro Gly Ser Gln Arg His Phe Ala	925	930	935	940

Thr Ser Cys His Gln Asp Glu Tyr Ser Gln Gln Ile Val Cys His Cys Arg Ala Gly Tyr
 945 950 955 960
 Thr Gly Leu Arg Cys Glu Ala Cys Ala Pro Gly His Phe Gly Asp Pro Ser Arg Pro Gly
 965 970 975 980
 Gly Arg Cys Gln Leu Cys Glu Cys Ser Gly Asn Ile Asp Pro Met Asp Pro Asp Ala Cys
 985 990 995 1000
 Asp Pro His Thr Gly Gln Cys Leu Arg Cys Leu His His Thr Glu Gly Pro His Cys Ala
 1005 1010 1015 1020
 His Cys Lys Pro Gly Phe His Gly Gln Ala Ala Arg Gln Ser Cys His Arg Cys Thr Cys
 1025 1030 1035 1040
 Asn Leu Leu Gly Thr Asn Pro Gln Gln Cys Pro Ser Pro Asp Gln Cys His Cys Asp Pro
 1045 1050 1055 1060
 Ser Ser Gly Gln Cys Pro Cys Leu Pro Asn Val Gln Gly Pro Ser Cys Asp Arg Cys Ala
 1065 1070 1075 1080
 Pro Asn Phe Trp Asn Leu Thr Ser Gly His Gly Cys Gln Pro Cys Ala Cys His Pro Ser
 1085 1090 1095 1100
 Arg Ala Arg Gly Pro Thr Cys Asn Glu Phe Thr Gly Gln Cys His Cys Arg Ala Gly Phe
 1105 1110 1115 1120
 Gly Gly Arg Thr Cys Ser Glu Cys Gln Glu Leu His Trp Gly Asp Pro Gly Leu Gln Cys
 1125 1130 1135 1140
 His Ala Cys Asp Cys Asp Ser Arg Gly Ile Asp Thr Pro Gln Cys His Arg Phe Thr Gly
 1145 1150 1155 1160
 His Cys Ser Cys Arg Pro Gly Val Ser Gly Val Arg Cys Asp Gln Cys Ala Arg Gly Phe
 1165 1170 1175 1180
 Ser Gly Ile Phe Pro Ala Cys His Pro Cys His Ala Cys Phe Gly Asp Trp Asp Arg Val
 1185 1190 1195 1200
 Val Gln Asp Leu Ala Ala Arg Thr Gln Arg Leu Glu Gln Arg Ala Gln Glu Leu Gln Gln
 1205 1210 1215 1220
 Thr Gly Val Leu Gly Ala Phe Glu Ser Ser Phe Trp His Met Gln Glu Lys Leu Gly Ile
 1225 1230 1235 1240
 Val Gln Gly Ile Val Gly Ala Arg Asn Thr Ser Ala Ala Ser Thr Ala Gln Leu Val Glu
 1245 1250 1255 1260
 Ala Thr Glu Glu Leu Arg Arg Glu Ile Gly Glu Ala Thr Glu His Leu Thr Gln Leu Glu
 1265 1270 1275 1280
 Ala Asp Leu Thr Asp Val Gln Asp Glu Asn Phe Asn Ala Asn His Ala Leu Ser Gly Leu
 1285 1290 1295 1300
 Glu Arg Asp Arg Leu Ala Leu Asn Leu Thr Leu Arg Gln Leu Asp Gln His Leu Asp Leu
 1305 1310 1315 1320
 Leu Lys His Ser Asn Phe Leu Gly Ala Tyr Asp Ser Ile Arg His Ala His Ser Gln Ser
 1325 1330 1335 1340
 Ala Glu Ala Glu Arg Arg Ala Asn Thr Ser Ala Leu Ala Val Pro Ser Pro Val Ser Asn
 1345 1350 1355 1360
 Ser Ala Ser Ala Arg His Arg Thr Glu Ala Leu Met Asp Ala Gln Lys Glu Asp Phe Asn
 1365 1370 1375 1380
 Ser Lys His Met Ala Asn Gln Arg Ala Leu Gly Lys Leu Ser Ala His Thr His Thr Leu
 1385 1390 1395 1400
 Ser Leu Thr Asp Ile Asn Glu Leu Val Cys Gly Ala Pro Gly Asp Ala Pro Cys Ala Thr
 1405 1410 1415 1420
 Ser Pro Cys Gly Gly Ala Gly Cys Arg Asp Glu Asp Gly Gln Pro Arg Cys Gly Gly Leu
 1425 1430 1435 1440
 Ser Cys Asn Gly Ala Ala Ala Thr Ala Asp Leu Ala Leu Gly Arg Ala Arg His Thr Gln
 1445 1450 1455 1460
 Ala Glu Leu Gln Arg Ala Leu Ala Glu Gly Gly Ser Ile Leu Ser Arg Val Ala Glu Thr
 1465 1470 1475 1480

Arg Arg Gln Ala Ser Glu Ala Gln Gln Arg Ala Gln Ala Ala Leu Asp Lys Ala Asn Ala	1485	1490	1495	1500
Ser Arg Gly Gln Val Glu Gln Ala Asn Gln Glu Leu Gln Glu Leu Ile Gln Ser Val Lys	1505	1510	1515	1520
Asp Phe Leu Asn Gln Glu Gly Ala Asp Pro Asp Ser Ile Glu Met Val Ala Thr Arg Val	1525	1530	1535	1540
Leu Glu Leu Ser Ile Pro Ala Ser Ala Glu Gln Ile Gln His Leu Ala Gly Ala Ile Ala	1545	1550	1555	1560
Glu Arg Val Arg Ser Leu Ala Asp Val Asp Ala Ile Leu Ala Arg Thr Val Gly Asp Val	1565	1570	1575	1580
Arg Arg Ala Glu Gln Leu Leu Gln Asp Ala Arg Arg Ala Arg Ser Trp Ala Glu Asp Glu	1585	1590	1595	1600
Lys Gln Lys Ala Glu Thr Val Gln Ala Ala Leu Glu Glu Ala Gln Arg Ala Gln Gly Ile	1605	1610	1615	1620
Ala Gln Gly Ala Ile Arg Gly Ala Val Ala Asp Thr Arg Asp Thr Glu Gln Thr Leu Tyr	1625	1630	1635	1640
Gln Val Gln Glu Arg Met Ala Gly Ala Glu Arg Ala Leu Ser Ser Ala Gly Glu Arg Ala	1645	1650	1655	1660
Arg Gln Leu Asp Ala Leu Leu Glu Ala Leu Lys Leu Lys Arg Ala Gly Asn Ser Leu Ala	1665	1670	1675	1680
Ala Ser Thr Ala Glu Glu Thr Ala Gly Ser Ala Gln Gly Arg Ala Gln Glu Ala Glu Gln	1685	1690	1695	1700
Leu Leu Arg Gly Pro Leu Gly Asp Gln Tyr Gln Thr Val Lys Ala Leu Ala Glu Arg Lys	1705	1710	1715	1720
Ala Gln Gly Val Leu Ala Ala Gln Ala Arg Ala Glu Gln Leu Arg Asp Glu Ala Arg Asp	1725	1730	1735	1740
Leu Leu Gln Ala Ala Gln Asp Lys Leu Gln Arg Leu Gln Glu Leu Glu Gly Thr Tyr Glu	1745	1750	1755	1760
Glu Asn Glu Arg Ala Leu Glu Ser Lys Ala Ala Gln Leu Asp Gly Leu Glu Ala Arg Met	1765	1770	1775	1780
Arg Ser Val Leu Gln Ala Ile Asn Leu Gln Val Gln Ile Tyr Asn Thr Cys Gln	1785	1790	1795	

INFORMATION FOR SEQ ID NO: 10:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 1607 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P02468;

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 10:

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Met Thr Gly Gly Gly Arg Ala Ala Leu Ala Leu Gln Pro Arg Gly Arg Leu Trp Pro Leu
 1           5           10           15           20
Leu Ala Val Leu Ala Ala Val Ala Gly Cys Val Arg Ala Ala Met Asp Glu Cys Ala Asp
          25           30           35           40
Glu Gly Gly Arg Pro Gln Arg Cys Met Pro Glu Phe Val Asn Ala Ala Phe Asn Val Thr
          45           50           55           60
Val Val Ala Thr Asn Thr Cys Gly Thr Pro Pro Glu Glu Tyr Cys Val Gln Thr Gly Val
          65           70           75           80
Thr Gly Val Thr Lys Ser Cys His Leu Cys Asp Ala Gly Gln Gln His Leu Gln His Gly
          85           90           95          100
Ala Ala Phe Leu Thr Asp Tyr Asn Asn Gln Ala Asp Thr Thr Trp Trp Gln Ser Gln Thr
          105          110          115          120
Met Leu Ala Gly Val Gln Tyr Pro Asn Ser Ile Asn Leu Thr Leu His Leu Gly Lys Ala
          125          130          135          140
Phe Asp Ile Thr Tyr Val Arg Leu Lys Phe His Thr Ser Arg Pro Glu Ser Phe Ala Ile
          145          150          155          160
Tyr Lys Arg Thr Arg Glu Asp Gly Pro Trp Ile Pro Tyr Gln Tyr Tyr Ser Gly Ser Cys
          165          170          175          180
Glu Asn Thr Tyr Ser Lys Ala Asn Arg Gly Phe Ile Arg Thr Gly Gly Asp Glu Gln Gln
          185          190          195          200
Ala Leu Cys Thr Asp Glu Phe Ser Asp Ile Ser Pro Leu Thr Gly Gly Asn Val Ala Phe
          205          210          215          220
Ser Thr Leu Glu Gly Arg Pro Ser Ala Tyr Asn Phe Asp Asn Ser Pro Val Leu Gln Glu
          225          230          235          240
Trp Val Thr Ala Thr Asp Ile Arg Val Thr Leu Asn Arg Leu Asn Thr Phe Gly Asp Glu
          245          250          255          260
Val Phe Asn Glu Pro Lys Val Leu Lys Ser Tyr Tyr Tyr Ala Ile Ser Asp Phe Ala Val
          265          270          275          280
Gly Gly Arg Cys Lys Cys Asn Gly His Ala Ser Glu Cys Val Lys Asn Glu Phe Asp Lys
          285          290          295          300
Leu Met Cys Asn Cys Lys His Asn Thr Tyr Gly Val Asp Cys Glu Lys Cys Leu Pro Phe
          305          310          315          320
Phe Asn Asp Arg Pro Trp Arg Arg Ala Thr Ala Glu Ser Ala Ser Glu Ser Leu Pro Cys
          325          330          335          340
Asp Cys Asn Gly Arg Ser Gln Glu Cys Tyr Phe Asp Pro Glu Leu Tyr Arg Ser Thr Gly
          345          350          355          360
His Gly Gly His Cys Thr Asn Cys Arg Asp Asn Thr Asp Gly Ala Lys Cys Glu Arg Cys
          365          370          375          380
Arg Glu Asn Phe Phe Arg Leu Gly Asn Thr Glu Ala Cys Ser Pro Cys His Cys Ser Pro
          385          390          395          400

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Val Gly Ser Leu	Ser Thr Gln Cys Asp	Ser Tyr Gly Arg Cys	Ser Cys Lys Pro Gly	Val
405	410	415	420	
Met Gly Asp Lys	Cys Asp Arg Cys Gln	Pro Gly Phe His Ser	Leu Thr Glu Ala Gly	Cys
425	430	435	440	
Arg Pro Cys Ser	Cys Asp Leu Arg Gly	Ser Thr Asp Glu Cys	Asn Val Glu Thr Gly	Arg
445	450	455	460	
Cys Val Cys Lys	Asp Asn Val Glu Gly	Phe Asn Cys Glu Arg	Cys Lys Pro Gly Phe	Phe
465	470	475	480	
Asn Leu Glu Ser	Ser Asn Pro Lys Gly	Cys Thr Pro Cys Phe	Cys Phe Gly His Ser	Ser
485	490	495	500	
Val Cys Thr Asn	Ala Val Gly Tyr Ser	Val Tyr Asp Ile Ser	Ser Thr Phe Gln Ile	Asp
505	510	515	520	
Glu Asp Gly Trp	Arg Val Glu Gln Arg	Asp Gly Ser Glu Ala	Ser Leu Glu Trp Ser	Ser
525	530	535	540	
Asp Arg Gln Asp	Ile Ala Val Ile Ser	Asp Ser Tyr Phe Pro	Arg Tyr Phe Ile Ala	Pro
545	550	555	560	
Val Lys Phe Leu	Gly Asn Gln Val Leu	Ser Tyr Gly Gln Asn	Leu Ser Phe Ser Phe	Arg
565	570	575	580	
Val Asp Arg Arg	Asp Thr Arg Leu Ser	Ala Glu Asp Leu Val	Leu Glu Gly Ala Gly	Leu
585	590	595	600	
Arg Val Ser Val	Pro Leu Ile Ala Gln	Gly Asn Ser Tyr Pro	Ser Glu Thr Thr Val	Lys
605	610	615	620	
Tyr Ile Phe Arg	Leu His Glu Ala Thr	Asp Tyr Pro Trp Arg	Pro Ala Leu Ser Pro	Phe
625	630	635	640	
Glu Phe Gln Lys	Leu Leu Asn Asn Leu	Thr Ser Ile Lys Ile	Arg Gly Thr Tyr Ser	Glu
645	650	655	660	
Arg Thr Ala Gly	Tyr Leu Asp Asp Val	Thr Leu Gln Ser Ala	Arg Pro Gly Pro Gly	Val
665	670	675	680	
Pro Ala Thr Trp	Val Glu Ser Cys Thr	Cys Pro Val Gly Tyr	Gly Gly Gln Phe Cys	Glu
685	690	695	700	
Thr Cys Leu Pro	Gly Tyr Arg Arg Glu	Thr Pro Ser Leu Gly	Pro Tyr Ser Pro Cys	Val
705	710	715	720	
Leu Cys Thr Cys	Asn Gly His Ser Glu	Thr Cys Asp Pro Glu	Thr Gly Val Cys Asp	Cys
725	730	735	740	
Arg Asp Asn Thr	Ala Gly Pro His Cys	Glu Lys Cys Ser Asp	Gly Tyr Tyr Gly Asp	Ser
745	750	755	760	
Thr Leu Gly Thr	Ser Ser Asp Cys Gln	Pro Cys Pro Cys Pro	Gly Gly Ser Ser Cys	Ala
765	770	775	780	
Ile Val Pro Lys	Thr Lys Glu Val Val	Cys Thr His Cys Pro	Thr Gly Thr Ala Gly	Lys
785	790	795	800	
Arg Cys Glu Leu	Cys Asp Asp Gly Tyr	Phe Gly Asp Pro Leu	Gly Ser Asn Gly Pro	Val
805	810	815	820	
Arg Leu Cys Arg	Pro Cys Gln Cys Asn	Asp Asn Ile Asp Pro	Asn Ala Val Gly Asn	Cys
825	830	835	840	
Asn Arg Leu Thr	Gly Glu Cys Leu Lys	Cys Ile Tyr Asn Thr	Ala Gly Phe Tyr Cys	Asp
845	850	855	860	
Arg Cys Lys Glu	Gly Phe Phe Gly Asn	Pro Leu Ala Pro Asn	Pro Ala Asp Lys Cys	Lys
865	870	875	880	
Ala Cys Ala Cys	Asn Pro Tyr Gly Thr	Val Gln Gln Gln Ser	Ser Cys Asn Pro Val	Thr
885	890	895	900	
Gly Gln Cys Gln	Cys Leu Pro His Val	Ser Gly Arg Asp Cys	Gly Thr Cys Asp Pro	Gly
905	910	915	920	
Tyr Tyr Asn Leu	Gln Ser Gly Gln Gly	Cys Glu Arg Cys Asp	Cys His Ala Leu Gly	Ser
925	930	935	940	

Thr Asn Gly Gln Cys Asp Ile Arg Thr Gly Gln Cys Glu Cys Gln Pro Gly Ile Thr Gly
 945 950 955 960
 Gln His Cys Glu Arg Cys Glu Thr Asn His Phe Gly Phe Gly Pro Glu Gly Cys Lys Pro
 965 970 975 980
 Cys Asp Cys His His Glu Gly Ser Leu Ser Leu Gln Cys Lys Asp Asp Gly Arg Cys Glu
 985 990 995 1000
 Cys Arg Glu Gly Phe Val Gly Asn Arg Cys Asp Gln Cys Glu Glu Asn Tyr Phe Tyr Asn
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 Arg Ser Trp Pro Gly Cys Gln Glu Cys Pro Ala Cys Tyr Arg Leu Val Lys Asp Lys Ala
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 Ala Glu His Arg Val Lys Leu Gln Glu Leu Glu Ser Leu Ile Ala Asn Leu Gly Thr Gly
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 Thr Asp Leu Leu Arg Glu Ala Gln Glu Val Lys Asp Val Asp Gln Asn Leu Met Asp Arg
 1085 1090 1095 1100
 Leu Gln Arg Val Asn Ser Ser Leu His Ser Gln Ile Ser Arg Leu Gln Asn Ile Arg Asn
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 Thr Ile Glu Glu Thr Gly Ile Leu Ala Glu Arg Ala Arg Ser Arg Val Glu Ser Thr Glu
 1125 1130 1135 1140
 Gln Leu Ile Glu Ile Ala Ser Arg Glu Leu Glu Lys Ala Lys Met Ala Ala Ala Asn Val
 1145 1150 1155 1160
 Ser Ile Thr Gln Pro Glu Ser Thr Gly Glu Pro Asn Asn Met Thr Leu Leu Ala Glu Glu
 1165 1170 1175 1180
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 1185 1190 1195 1200
 Thr Ala Asn Glu Thr Ser Ala Glu Ala Tyr Asn Leu Leu Leu Arg Thr Leu Ala Gly Glu
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 Asn Gln Thr Ala Leu Glu Ile Glu Glu Leu Asn Arg Lys Tyr Glu Gln Ala Lys Asn Ile
 1225 1230 1235 1240
 Ser Gln Asp Leu Glu Lys Gln Ala Ala Arg Val His Glu Glu Ala Lys Arg Ala Gly Asp
 1245 1250 1255 1260
 Lys Ala Val Glu Ile Tyr Ala Ser Val Ala Gln Leu Thr Pro Val Asp Ser Glu Ala Leu
 1265 1270 1275 1280
 Glu Asn Glu Ala Asn Lys Ile Lys Lys Glu Ala Ala Asp Leu Asp Arg Leu Ile Asp Gln
 1285 1290 1295 1300
 Lys Leu Lys Asp Tyr Glu Asp Leu Arg Glu Asp Met Arg Gly Lys Glu His Glu Val Lys
 1305 1310 1315 1320
 Asn Leu Leu Glu Lys Gly Lys Ala Glu Gln Gln Thr Ala Asp Gln Leu Leu Ala Arg Ala
 1325 1330 1335 1340
 Asp Ala Ala Lys Ala Leu Ala Glu Glu Ala Ala Lys Lys Gly Arg Ser Thr Leu Gln Glu
 1345 1350 1355 1360
 Ala Asn Asp Ile Leu Asn Asn Leu Lys Asp Phe Asp Arg Arg Val Asn Asp Asn Lys Thr
 1365 1370 1375 1380
 Ala Ala Glu Glu Ala Leu Arg Arg Ile Pro Ala Ile Asn Arg Thr Ile Ala Glu Ala Asn
 1385 1390 1395 1400
 Glu Lys Thr Arg Glu Ala Gln Leu Ala Leu Gly Asn Ala Ala Ala Asp Ala Thr Glu Ala
 1405 1410 1415 1420
 Lys Asn Lys Ala His Glu Ala Glu Arg Ile Ala Ser Ala Val Gln Lys Asn Ala Thr Ser
 1425 1430 1435 1440
 Thr Lys Ala Asp Ala Glu Arg Thr Phe Gly Glu Val Thr Asp Leu Asp Asn Glu Val Asn
 1445 1450 1455 1460
 Gly Met Leu Arg Gln Leu Glu Glu Ala Glu Asn Glu Leu Lys Arg Lys Gln Asp Asp Ala
 1465 1470 1475 1480

Asp Gln Asp Met Met Met Ala Gly Met Ala Ser Gln Ala Ala Gln Glu Ala Glu Leu Asn	1485	1490	1495	1500
Ala Arg Lys Ala Lys Asn Ser Val Ser Ser Leu Leu Ser Gln Leu Asn Asn Leu Leu Asp	1505	1510	1515	1520
Gln Leu Gly Gln Leu Asp Thr Val Asp Leu Asn Lys Leu Asn Glu Ile Glu Gly Ser Leu	1525	1530	1535	1540
Asn Lys Ala Lys Asp Glu Met Lys Ala Ser Asp Leu Asp Arg Lys Val Ser Asp Leu Glu	1545	1550	1555	1560
Ser Glu Ala Arg Lys Gln Glu Ala Ala Ile Met Asp Tyr Asn Arg Asp Ile Ala Glu Ile	1565	1570	1575	1580
Ile Lys Asp Ile His Asn Leu Glu Asp Ile Lys Lys Thr Leu Pro Thr Gly Cys Phe Asn	1585	1590	1595	1600
Thr Pro Ser Ile Glu Lys Pro	1605			

INFORMATION FOR SEQ ID NO: 11:

SEQUENCE CHARACTERISTICS

(A) LENGTH: 1609 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(E) AMINO ACID NUMBERING ACCORDING TO TRANSLATION OF
GENEBANK ACCESSION NUMBER P11047;

MOLECULAR TYPE: PROTEIN

SEQUENCE DESCRIPTION: SEQ ID NO 11:

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Leu	Ala	Val	Leu	Ala	Ala	Ala	Ala	Ala	Ala	Gly	Cys	Ala	Gln	Ala	Ala	Met	Asp	Glu	Cys	25	30	35	40	
Thr	Asp	Glu	Gly	Gly	Arg	Pro	Gln	Arg	Cys	Met	Pro	Glu	Phe	Val	Asn	Ala	Ala	Phe	Asn	45	50	55	60	
Val	Thr	Val	Val	Ala	Thr	Asn	Thr	Cys	Gly	Thr	Pro	Pro	Glu	Glu	Tyr	Cys	Val	Gln	Thr	65	70	75	80	
Gly	Val	Thr	Gly	Val	Thr	Lys	Ser	Cys	His	Leu	Cys	Asp	Ala	Gly	Gln	Pro	His	Leu	Gln	85	90	95	100	
His	Gly	Ala	Ala	Phe	Leu	Thr	Asp	Tyr	Asn	Asn	Gln	Ala	Asp	Thr	Thr	Trp	Trp	Gln	Ser	105	110	115	120	
Gln	Thr	Met	Leu	Ala	Gly	Val	Gln	Tyr	Pro	Ser	Ser	Ile	Asn	Leu	Thr	Leu	His	Leu	Gly	125	130	135	140	
Lys	Ala	Phe	Asp	Ile	Thr	Tyr	Val	Arg	Leu	Lys	Phe	His	Thr	Ser	Arg	Pro	Glu	Ser	Phe	145	150	155	160	
Ala	Ile	Tyr	Lys	Arg	Thr	Arg	Glu	Asp	Gly	Pro	Trp	Ile	Pro	Tyr	Gln	Tyr	Tyr	Ser	Gly	165	170	175	180	
Ser	Cys	Glu	Asn	Thr	Tyr	Ser	Lys	Ala	Asn	Arg	Gly	Phe	Ile	Arg	Thr	Gly	Gly	Asp	Glu	185	190	195	200	
Gln	Gln	Ala	Leu	Cys	Thr	Asp	Glu	Phe	Ser	Asp	Phe	Ser	Pro	Leu	Thr	Gly	Gly	Asn	Val	205	210	215	220	
Ala	Phe	Ser	Thr	Leu	Glu	Gly	Arg	Pro	Ser	Ala	Tyr	Asn	Phe	Asp	Asn	Ser	Pro	Val	Leu	225	230	235	240	
Gln	Glu	Trp	Val	Thr	Ala	Thr	Asp	Ile	Arg	Val	Thr	Leu	Asn	Arg	Leu	Asn	Thr	Phe	Gly	245	250	255	260	
Asp	Glu	Val	Phe	Asn	Asp	Pro	Lys	Val	Leu	Lys	Ser	Tyr	Tyr	Tyr	Ala	Ile	Ser	Asp	Phe	265	270	275	280	
Ala	Val	Gly	Gly	Arg	Cys	Lys	Cys	Asn	Gly	His	Ala	Ser	Glu	Cys	Met	Lys	Asn	Glu	Phe	285	290	295	300	
Asp	Lys	Leu	Val	Cys	Asn	Cys	Lys	His	Asn	Thr	Tyr	Gly	Val	Asp	Cys	Glu	Lys	Cys	Leu	305	310	315	320	
Pro	Phe	Phe	Asn	Asp	Arg	Pro	Trp	Arg	Arg	Ala	Thr	Ala	Glu	Ser	Ala	Ser	Glu	Cys	Leu	325	330	335	340	
Pro	Cys	Asp	Cys	Asn	Gly	Arg	Ser	Gln	Glu	Cys	Tyr	Phe	Asp	Pro	Glu	Leu	Tyr	Arg	Ser	345	350	355	360	
Thr	Gly	His	Gly	Gly	His	Cys	Thr	Asn	Cys	Gln	Asp	Asn	Thr	Asp	Gly	Ala	His	Cys	Glu	365	370	375	380	
Arg	Cys	Arg	Glu	Asn	Phe	Phe	Arg	Leu	Gly	Asn	Asn	Glu	Ala	Cys	Ser	Ser	Cys	His	Cys	385	390	395	400	

Ser Pro Val Gly	Ser Leu Ser Thr Gln	Cys Asp Ser Tyr Gly	Arg Cys Ser Cys Lys	Pro
405		410	415	420
Gly Val Met Gly	Asp Lys Cys Asp Arg	Cys Gln Pro Gly Phe	His Ser Leu Thr Glu	Ala
425		430	435	440
Gly Cys Arg Pro	Cys Ser Cys Asp Pro	Ser Gly Ser Ile Asp	Glu Cys Asn Val Glu	Thr
445		450	455	460
Gly Arg Cys Val	Cys Lys Asp Asn Val	Glu Gly Phe Asn Cys	Glu Arg Cys Lys Pro	Gly
465		470	475	480
Phe Phe Asn Leu	Glu Ser Ser Asn Pro	Arg Gly Cys Thr Pro	Cys Phe Cys Phe Gly	His
485		490	495	500
Ser Ser Val Cys	Thr Asn Ala Val Gly	Tyr Ser Val Tyr Ser	Ile Ser Ser Thr Phe	Gln
505		510	515	520
Ile Asp Glu Asp	Gly Trp Arg Ala Glu	Gln Arg Asp Gly Ser	Glu Ala Ser Leu Glu	Trp
525		530	535	540
Ser Ser Glu Arg	Gln Asp Ile Ala Val	Ile Ser Asp Ser Tyr	Phe Pro Arg Tyr Phe	Ile
545		550	555	560
Ala Pro Ala Lys	Phe Leu Gly Lys Gln	Val Leu Ser Tyr Gly	Gln Asn Leu Ser Phe	Ser
565		570	575	580
Phe Arg Val Asp	Arg Arg Asp Thr Arg	Leu Ser Ala Glu Asp	Leu Val Leu Glu Gly	Ala
585		590	595	600
Gly Leu Arg Val	Ser Val Pro Leu Ile	Ala Gln Gly Asn Ser	Tyr Pro Ser Glu Thr	Thr
605		610	615	620
Val Lys Tyr Val	Phe Arg Leu His Glu	Ala Thr Asp Tyr Pro	Trp Arg Pro Ala Leu	Thr
625		630	635	640
Pro Phe Glu Phe	Gln Lys Leu Leu Asn	Asn Leu Thr Ser Ile	Lys Ile Arg Gly Thr	Tyr
645		650	655	660
Ser Glu Arg Ser	Ala Gly Tyr Leu Asp	Asp Val Thr Leu Ala	Ser Ala Arg Pro Gly	Pro
665		670	675	680
Gly Val Pro Ala	Thr Trp Val Glu Ser	Cys Thr Cys Pro Val	Gly Tyr Gly Gly Gln	Phe
685		690	695	700
Cys Glu Met Cys	Leu Ser Gly Tyr Arg	Arg Glu Thr Pro Asn	Leu Gly Pro Tyr Ser	Pro
705		710	715	720
Cys Val Leu Cys	Ala Cys Asn Gly His	Ser Glu Thr Cys Asp	Pro Glu Thr Gly Val	Cys
725		730	735	740
Asn Cys Arg Asp	Asn Thr Ala Gly Pro	His Cys Glu Lys Cys	Ser Asp Gly Tyr Tyr	Gly
745		750	755	760
Asp Ser Thr Ala	Gly Thr Ser Ser Asp	Cys Gln Pro Cys Pro	Cys Pro Gly Gly Ser	Ser
765		770	775	780
Cys Ala Val Val	Pro Lys Thr Lys Glu	Val Val Cys Thr Asn	Cys Pro Thr Gly Thr	Thr
785		790	795	800
Gly Lys Arg Cys	Glu Leu Cys Asp Asp	Gly Tyr Phe Gly Asp	Pro Leu Gly Arg Asn	Gly
805		810	815	820
Pro Val Arg Leu	Cys Arg Leu Cys Gln	Cys Ser Asp Asn Ile	Asp Pro Asn Ala Val	Gly
825		830	835	840
Asn Cys Asn Arg	Leu Thr Gly Glu Cys	Leu Lys Cys Ile Tyr	Asn Thr Ala Gly Phe	Tyr
845		850	855	860
Cys Asp Arg Cys	Lys Asp Gly Phe Phe	Gly Asn Pro Leu Ala	Pro Asn Pro Ala Asp	Lys
865		870	875	880
Cys Lys Ala Cys	Asn Cys Asn Pro Tyr	Gly Thr Met Lys Gln	Gln Ser Ser Cys Asn	Pro
885		890	895	900
Val Thr Gly Gln	Cys Glu Cys Leu Pro	His Val Thr Gly Gln	Asp Cys Gly Ala Cys	Asp
905		910	915	920
Pro Gly Phe Tyr	Asn Leu Gln Ser Gly	Gln Gly Cys Glu Arg	Cys Asp Cys His Ala	Leu
925		930	935	940

Gly Ser Thr Asn Gly Gln Cys Asp Ile Arg Thr Gly Gln Cys Glu Cys Gln Pro Gly Ile
 945 950 955 960
 Thr Gly Gln His Cys Glu Arg Cys Glu Val Asn His Phe Gly Phe Gly Pro Glu Gly Cys
 965 970 975 980
 Lys Pro Cys Asp Cys His Pro Glu Gly Ser Leu Ser Leu Gln Cys Lys Asp Asp Gly Arg
 985 990 995 1000
 Cys Glu Cys Arg Glu Gly Phe Val Gly Asn Arg Cys Asp Gln Cys Glu Glu Asn Tyr Phe
 1005 1010 1015 1020
 Tyr Asn Arg Ser Trp Pro Gly Cys Gln Glu Cys Pro Ala Cys Tyr Arg Leu Val Lys Asp
 1025 1030 1035 1040
 Lys Val Ala Asp His Arg Val Lys Leu Gln Glu Leu Glu Ser Leu Ile Ala Asn Leu Gly
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 Thr Gly Asp Glu Met Val Thr Asp Gln Ala Phe Glu Asp Arg Leu Lys Glu Ala Glu Arg
 1065 1070 1075 1080
 Glu Val Met Asp Leu Leu Arg Glu Ala Gln Asp Val Lys Asp Val Asp Gln Asn Leu Met
 1085 1090 1095 1100
 Asp Arg Leu Gln Arg Val Asn Asn Thr Leu Ser Ser Gln Ile Ser Arg Leu Gln Asn Ile
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 Arg Asn Thr Ile Glu Glu Thr Gly Asn Leu Ala Glu Gln Ala Arg Ala His Val Glu Asn
 1125 1130 1135 1140
 Thr Glu Arg Leu Ile Glu Ile Ala Ser Arg Glu Leu Glu Lys Ala Lys Val Ala Ala Ala
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 1165 1170 1175 1180
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 Ala Lys Thr Ala Asn Asp Thr Ser Thr Glu Ala Tyr Asn Leu Leu Leu Arg Thr Leu Ala
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 Gly Glu Asn Gln Thr Ala Phe Glu Ile Glu Glu Leu Asn Arg Lys Tyr Glu Gln Ala Lys
 1225 1230 1235 1240
 Asn Ile Ser Gln Asp Leu Glu Lys Gln Ala Ala Arg Val His Glu Glu Ala Lys Arg Ala
 1245 1250 1255 1260
 Gly Asp Lys Ala Val Glu Ile Tyr Ala Ser Val Ala Gln Leu Ser Pro Leu Asp Ser Glu
 1265 1270 1275 1280
 Thr Leu Glu Asn Glu Ala Asn Asn Ile Lys Met Glu Ala Glu Asn Leu Glu Gln Leu Ile
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 Asp Gln Lys Leu Lys Asp Tyr Glu Asp Leu Arg Glu Asp Met Arg Gly Lys Glu Leu Glu
 1305 1310 1315 1320
 Val Lys Asn Leu Leu Glu Lys Gly Lys Thr Glu Gln Gln Thr Ala Asp Gln Leu Leu Ala
 1325 1330 1335 1340
 Arg Ala Asp Ala Ala Lys Ala Leu Ala Glu Glu Ala Ala Lys Lys Gly Arg Asp Thr Leu
 1345 1350 1355 1360
 Gln Glu Ala Asn Asp Ile Leu Asn Asn Leu Lys Asp Phe Asp Arg Arg Val Asn Asp Asn
 1365 1370 1375 1380
 Lys Thr Ala Ala Glu Glu Ala Leu Arg Lys Ile Pro Ala Ile Asn Gln Thr Ile Thr Glu
 1385 1390 1395 1400
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 Glu Ala Lys Asn Lys Ala His Glu Ala Glu Arg Ile Ala Ser Ala Val Gln Lys Asn Ala
 1425 1430 1435 1440
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 1445 1450 1455 1460
 Val Asn Asn Met Leu Lys Gln Leu Gln Glu Ala Glu Lys Glu Leu Lys Arg Lys Gln Asp
 1465 1470 1475 1480

Asp	Ala	Asp	Gln	Asp	Met	Met	Met	Ala	Gly	Met	Ala	Ser	Gln	Ala	Ala	Gln	Glu	Ala	Glu	1485	1490	1495	1500
Ile	Asn	Ala	Arg	Lys	Ala	Lys	Asn	Ser	Val	Thr	Ser	Leu	Leu	Ser	Ile	Ile	Asn	Asp	Leu	1505	1510	1515	1520
Leu	Glu	Gln	Leu	Gly	Gln	Leu	Asp	Thr	Val	Asp	Leu	Asn	Lys	Leu	Asn	Glu	Ile	Glu	Gly	1525	1530	1535	1540
Thr	Leu	Asn	Lys	Ala	Lys	Asp	Glu	Met	Lys	Val	Ser	Asp	Leu	Asp	Arg	Lys	Val	Ser	Asp	1545	1550	1555	1560
Leu	Glu	Asn	Glu	Ala	Lys	Lys	Gln	Glu	Ala	Ala	Ile	Met	Asp	Tyr	Asn	Arg	Asp	Ile	Glu	1565	1570	1575	1580
Glu	Ile	Met	Lys	Asp	Ile	Arg	Asn	Leu	Glu	Asp	Ile	Arg	Lys	Thr	Leu	Pro	Ser	Gly	Cys	1585	1590	1595	1600
Phe	Asn	Thr	Pro	Ser	Ile	Glu	Lys	Pro												1605			

CLAIMS

We claim:

1. A method for treating an amyloid disease in a patient, the method comprising administering to the patient a therapeutically effective amount of a polypeptide
5 having a conformational similarity to a fragment of a laminin protein.
2. The method of claim 1 wherein the conformational similarity is at least 70%.
3. The method of claim 1 wherein the conformational similarity is at least 90%.
4. The method of claim 1 wherein the polypeptide is synthesized to achieve said conformational similarity.
- 10 5. The method of claim 1 wherein said amyloid disease is Alzheimer's disease.
6. The method of claim 1 wherein said fragment is intact laminin.
7. The method of claim 1 wherein the laminin fragment is a laminin A chain.
8. The method of claim 7 wherein the laminin A chain is derived from mammals.
9. The method of claim 8 wherein the fragment comprises a polypeptide as set
15 forth in SEQ ID NO: 5 or a fragment thereof.
10. The method of claim 8 wherein the fragment comprises a polypeptide as set forth in SEQ ID NO: 4 or a fragment thereof.
11. The method of claim 1 wherein the laminin fragment includes a globular domain repeat within the laminin A chain or a fragment thereof.
- 20 12. The method of claim 11 wherein the globular repeats include the peptide sequence of SEQ ID NO: 3 or a fragment thereof.
13. The method of claim 11 wherein the globular repeats include the peptide sequence of SEQ ID NO: 2 or a fragment thereof.
14. The method of claim 11 wherein the laminin fragment includes the peptide
25 sequence of SEQ ID NO: 1 or a fragment thereof.

15. A method for the treatment of a patient having an identified clinical need to interfere with the pathological effects of amyloid, the method comprising: administering to the patient a therapeutically effective amount of a polypeptide selected from the group consisting of human laminin, mouse laminin, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO 4:, SEQ ID NO: 5, SEQ ID NO:6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO: 11, and fragments thereof.
16. A method to diagnose a disease or a susceptibility to a disease related to the levels of laminin or laminin-derived protein fragments, the method comprising determining levels of laminin or a particular laminin-derived protein fragment in a sample, whereby the levels are indicative of the presence of a disease, susceptibility to a disease, or progression of said disease.
17. The method of claim 16 wherein said disease is an amyloid disease.
18. The method of claim 16 wherein said laminin or laminin-derived protein fragments is selected from the group consisting of human laminin, mouse laminin, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO 4:, SEQ ID NO: 5, SEQ ID NO:6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO: 11, and fragments thereof.
19. The method of claim 16 wherein said laminin-derived protein fragment is a 130 kilodalton fragment detected by ligand blotting with biotinylated beta-amyloid protein (A β), and quantitated by scanning densitometry or by ELISA.
20. The method of claim 16 wherein the sample assayed is a biological fluid.
21. The method of claim 20 wherein the biological fluid is serum.
22. The method of claim 20 wherein the biological fluid is derived from humans.
23. A method of making an antibody, the method comprising producing antibodies from a peptide sequence within the 130 kilodalton A β -laminin binding fragment present in human biological fluids.

24. The method of claim 23 wherein antibody production comprises production of at least one type of antibody selected from the group consisting of polyclonal, monoclonal, chimeric antibodies, and anti-idiotypic antibodies.
25. The method of claim 23 wherein the peptide sequence is selected from the
5 group of SEQ ID's consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO 4:, SEQ ID NO: 5, SEQ ID NO:6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO: 11, and fragments thereof.
26. The method of claim 23 further comprising monitoring a biological fluid for the presence and extent of laminin and laminin-derived protein fragments as an indicator
10 for the extent of an amyloid disease.
27. A process for diagnosing a disease or a susceptibility to a disease related to an underexpression or overexpression of a polypeptide, comprising determining a mutation in a nucleic acid sequence encoding a polypeptide selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO 4:, SEQ ID
15 NO: 5, SEQ ID NO:6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO: 11, and fragments thereof.
28. The method of claim 23 further comprising radiolabelling the antibodies for radioimaging or in vivo diagnosis for detection of laminin and laminin-derived protein fragments.
- 20 29. A method for detection and quantification of laminin and laminin-derived fragments in biological fluids comprising a) allowing a first laminin or laminin-derived fragment antibody to bind to microtiter wells for a sufficient time to allow said binding, b) adding a quantity of biological fluid to the microtiter wells, c) incubating the biological fluid for sufficient time to allow binding of any laminin or
25 laminin-derived fragment in the biological fluid to the first antibody on the microtiter wells, d) adding a second labeled antibody to the microtiter wells wherein the second labelled antibody is against the laminin or laminin-derived fragment, but which is

against a different epitope than the first antibody, and allowing the second antibody to bind to any laminin or laminin-derived fragment captured by the first antibody, and e) detecting bound materials using an appropriate substrate or label.

30. A composition of matter comprising a purified laminin polypeptide fragment
5 that is capable of binding to A β amyloid protein, wherein the laminin polypeptide fragment has an A β binding site within a globular repeating domain of laminin A chain.
31. The laminin polypeptide fragment of claim 30 wherein the fragment comprises a 55 kilodalton elastase-resistant laminin polypeptide fragment.
- 10 32. The laminin polypeptide fragment of claim 31 wherein the fragment comprises a 55 kilodalton laminin polypeptide fragment that is produced using a protease from the group of proteases consisting of trypsin and elastase.
33. The laminin polypeptide fragment of claim 32 wherein the fragment comprises SEQ ID NO: 5.
- 15 34. A method of in vivo inhibition of A β amyloidosis comprising: a) introducing a vector comprising the DNA sequence encoding a laminin polypeptide fragment that is capable of binding to A β amyloid protein, wherein the laminin polypeptide fragment has an A β binding site within a globular repeating domain of laminin A chain, b) producing said laminin polypeptide fragment in vivo to inhibit A β amyloidosis.
- 20 35. A method of in vivo inhibition of A β amyloidosis comprising: a) introducing a vector comprising the DNA sequence encoding the polypeptide of SEQ ID NO: 3 or a fragment thereof, b) producing a peptide fragment having the polypeptide sequence of SEQ ID NO: 3 in vivo to inhibit A β amyloidosis.
36. The method of claim 1 wherein the fragment of laminin protein is an amyloid
25 binding fragment of laminin protein

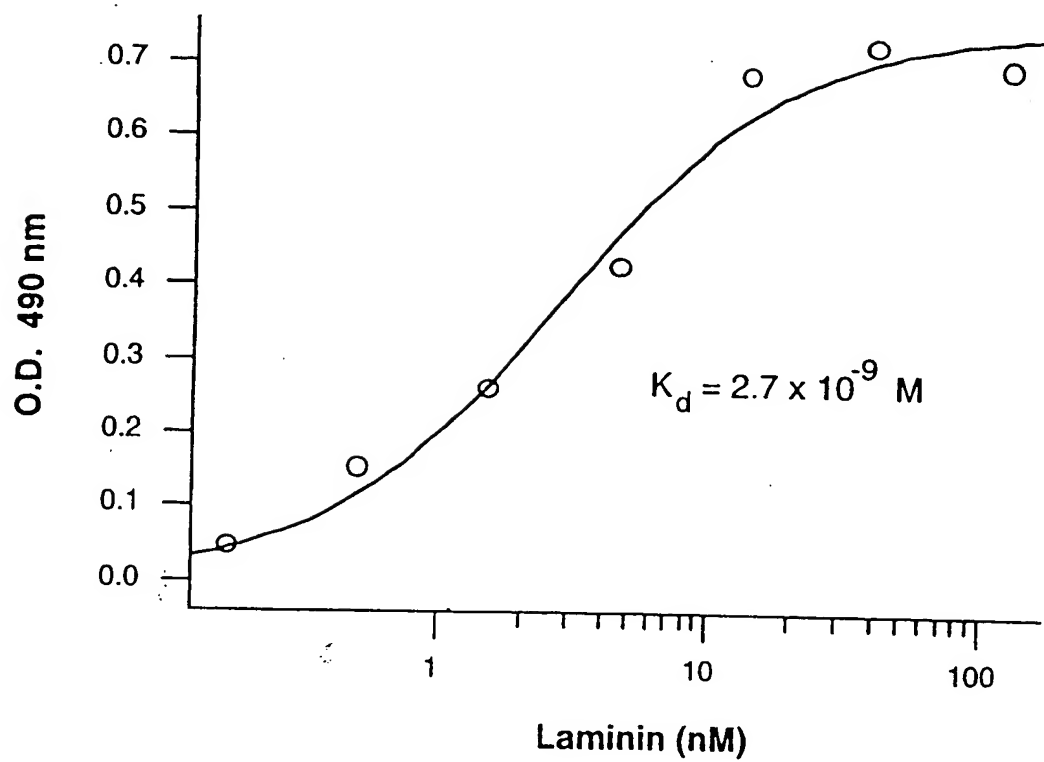


FIGURE 1

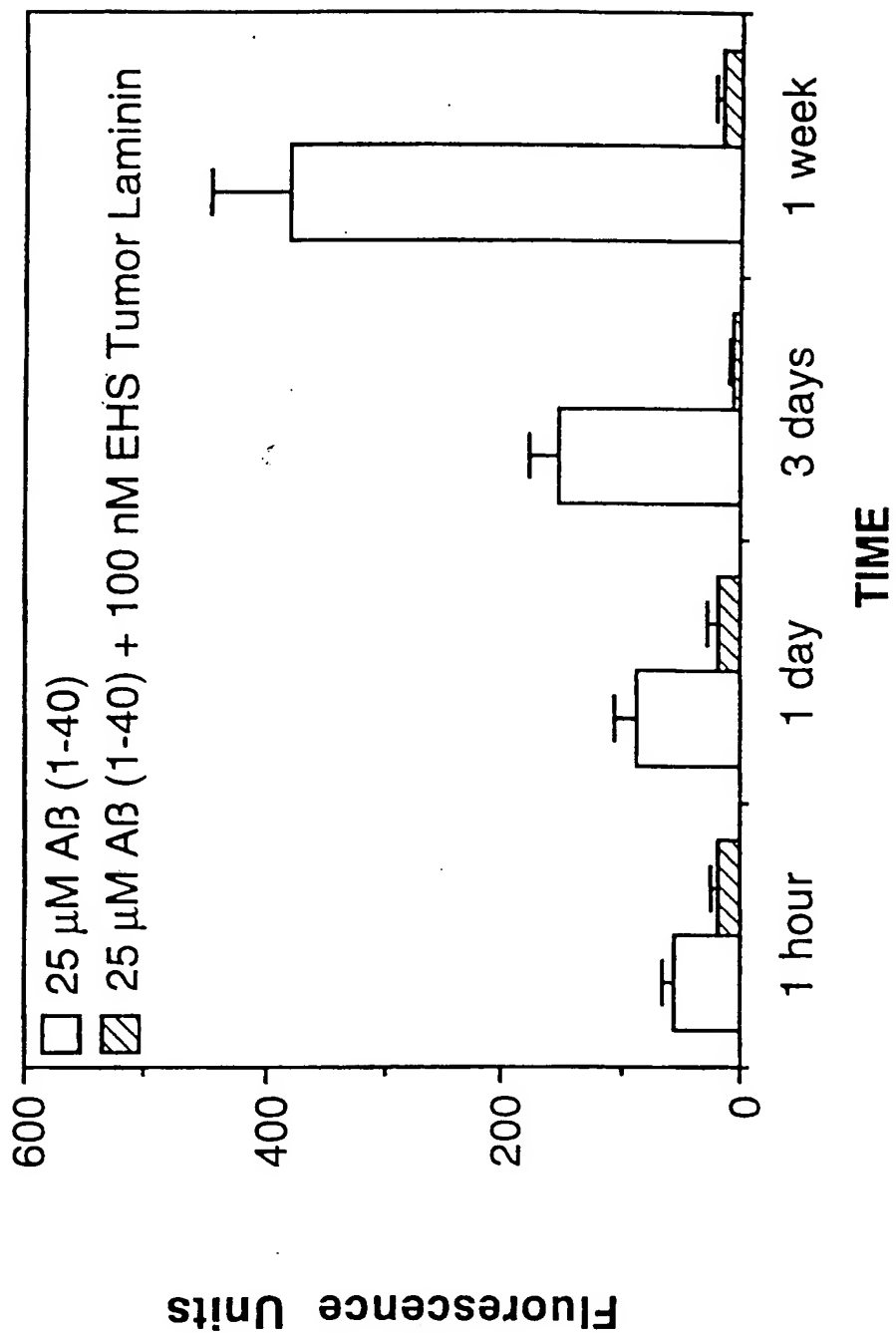


FIGURE 2

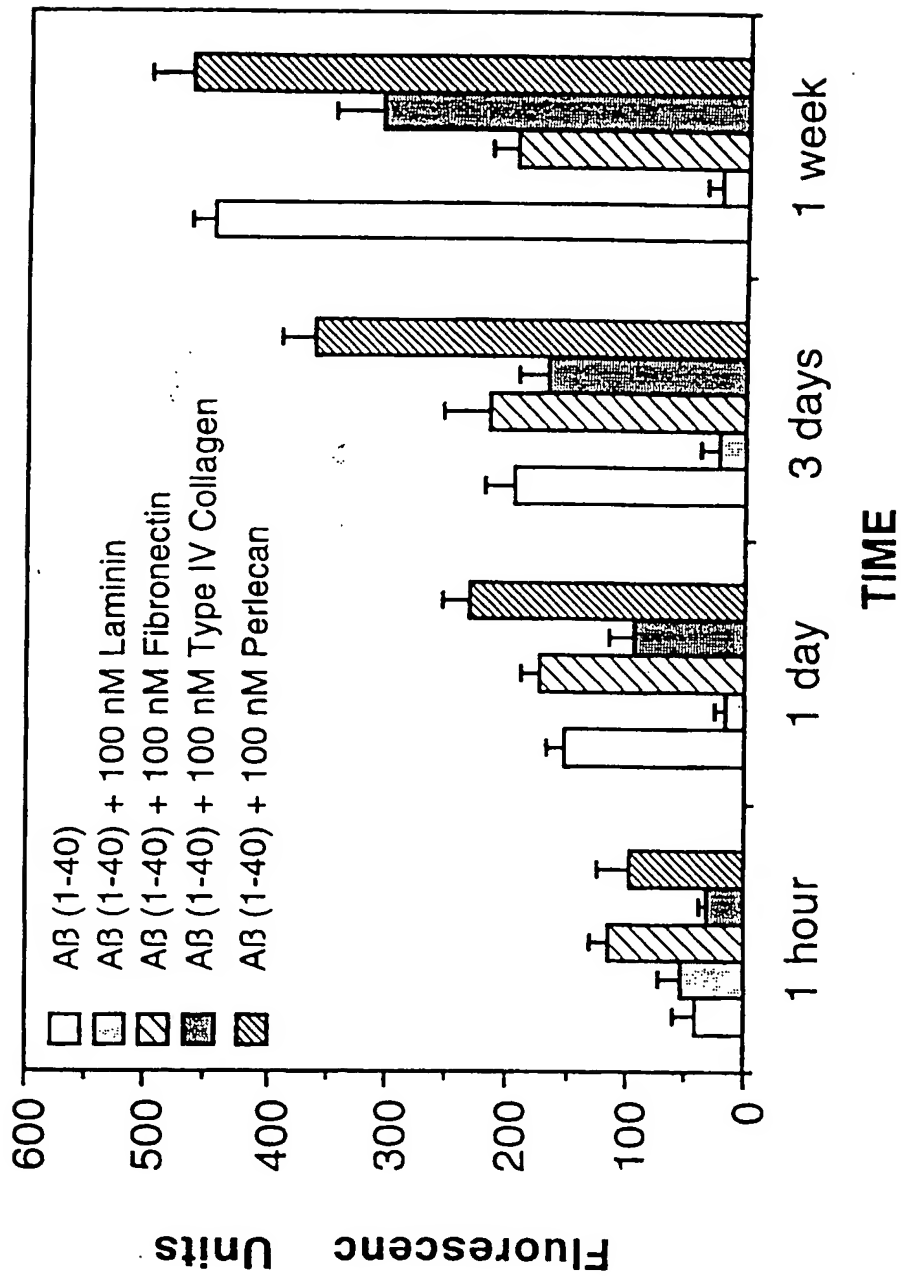


FIGURE 3

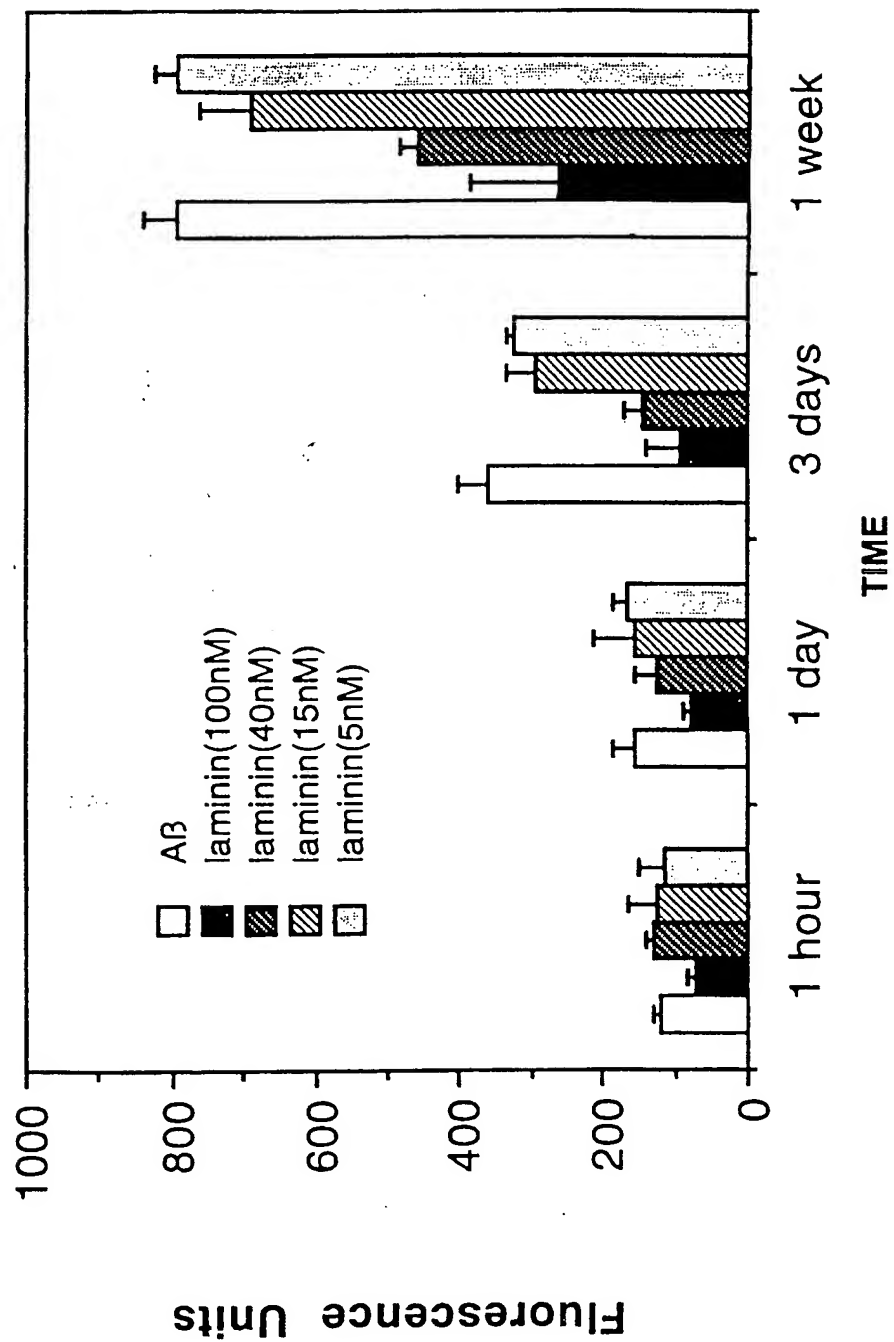


FIGURE 4

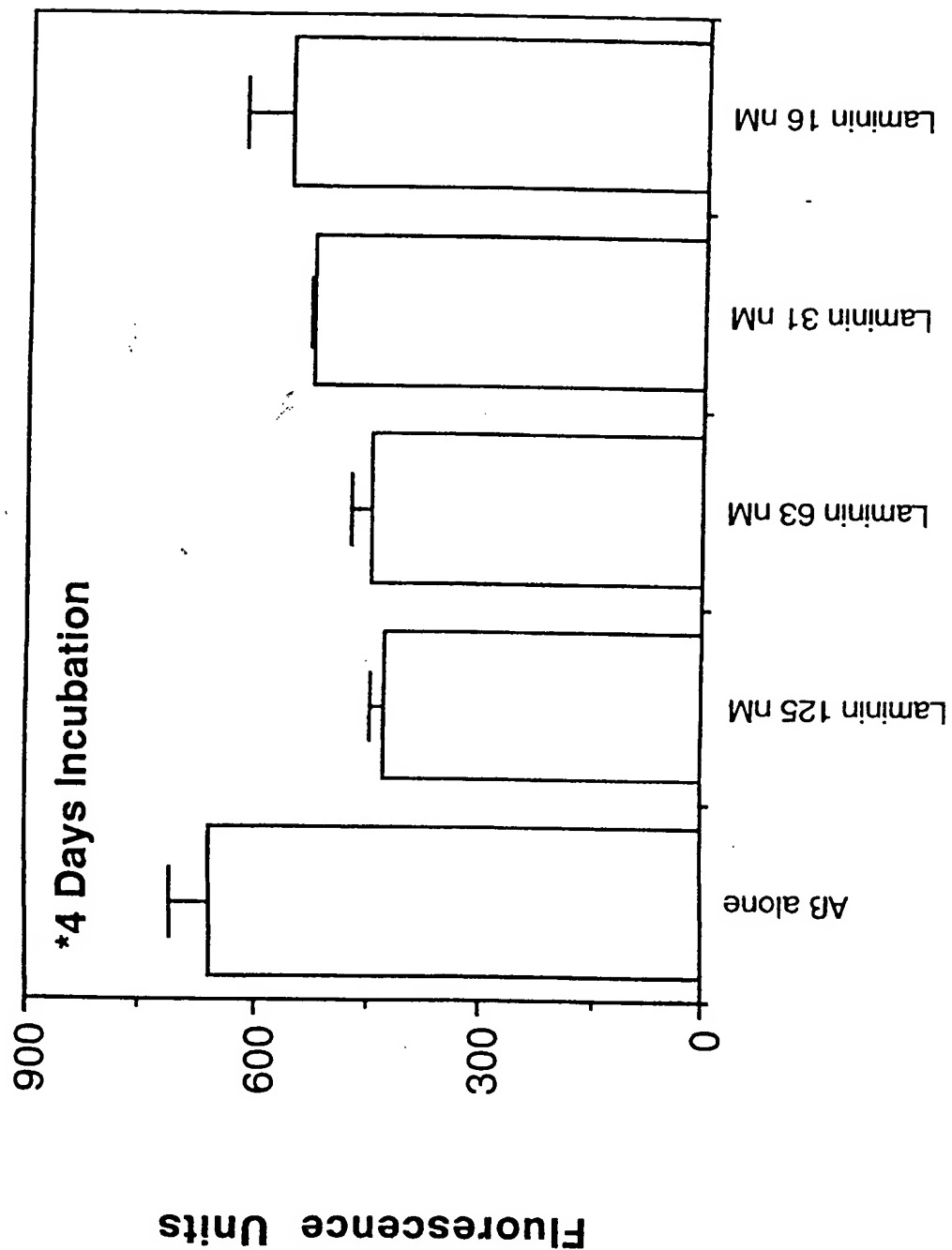


FIGURE 5

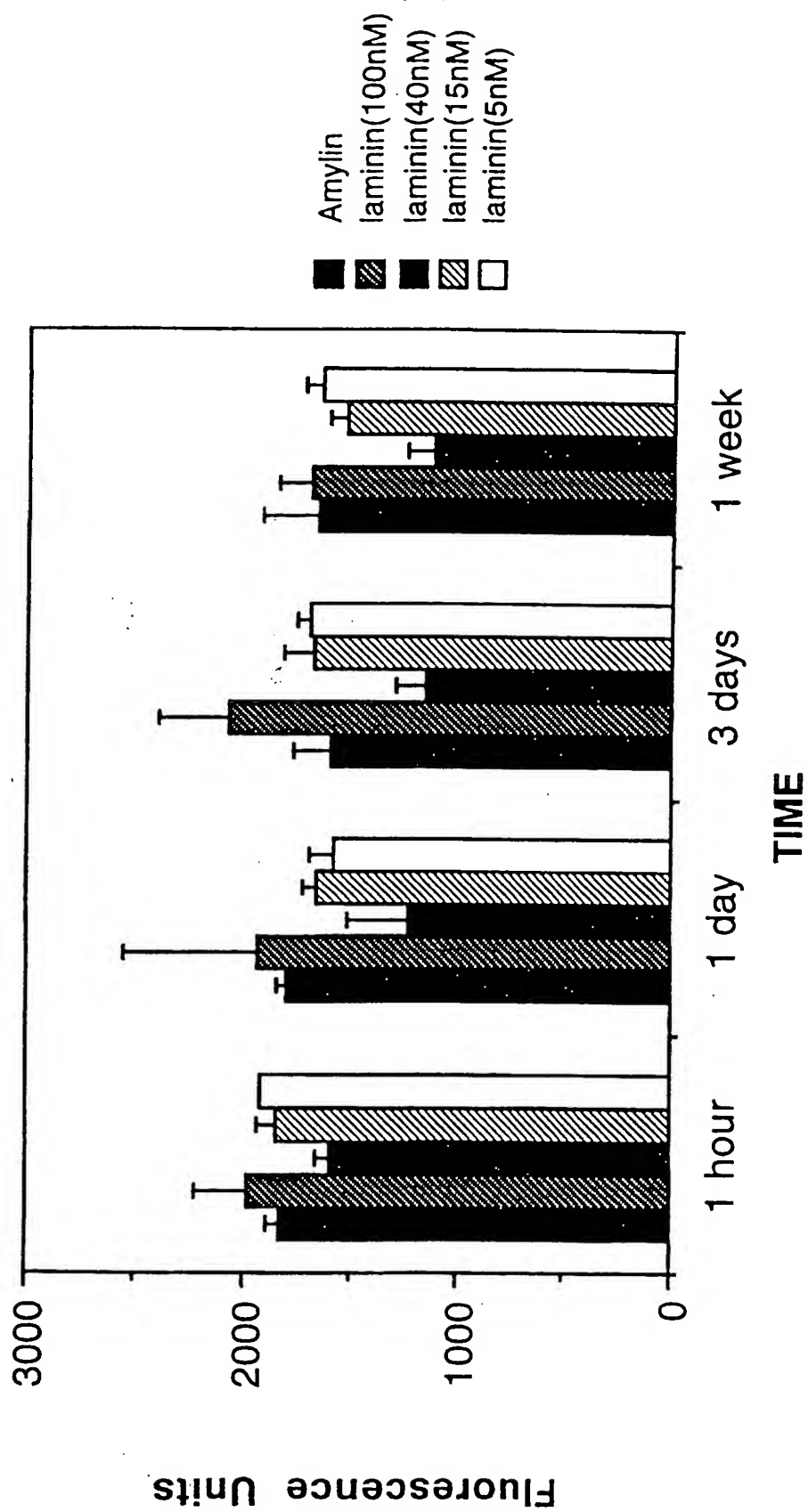


FIGURE 6

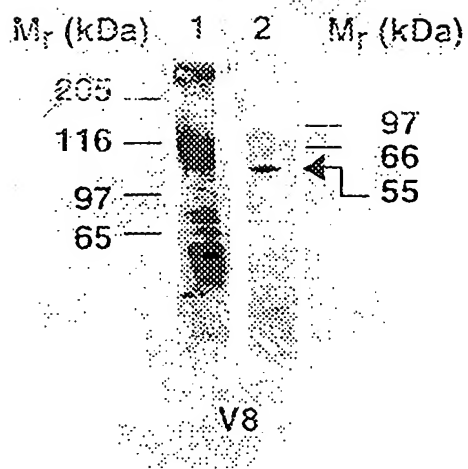


FIGURE 7

SUBSTITUTE SHEET (RULE 28)

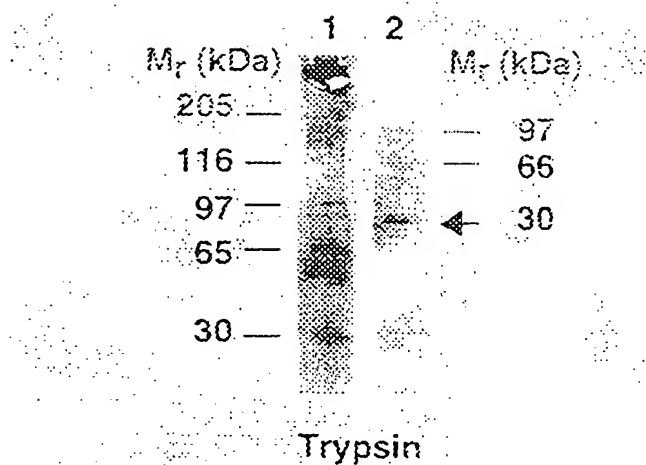


FIGURE 8

SUBSTITUTE SHEET (RULE 26)

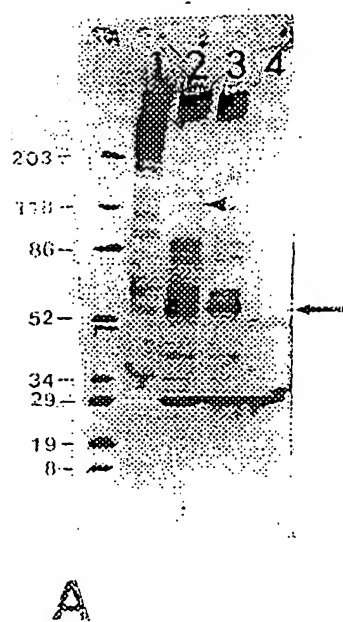


FIGURE 9a

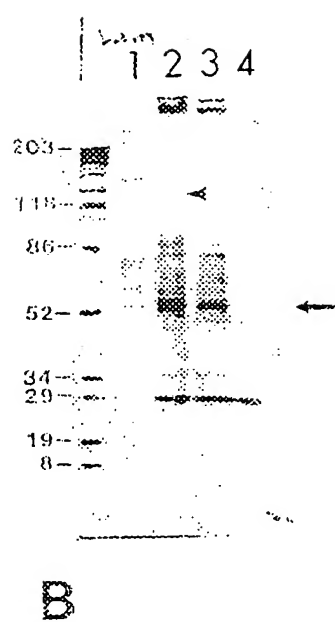


FIGURE 9b

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10/14

SEQUENCE

MRGSGTGAAL	LVLLASVLWV	TVRSQQRGLF	PAILNLATNA	HISANATCGE	KGPEMFCKLV
EHVPGRPVRH	AQCRVCDGNS	TNPRERHPIS	HAIDGTNNWW	QSPSIQNGRE	YHWVTVTLDL
RQVFQVAYII	IKAANAPRPG	NWILERSVDG	VKFKPWQYYA	VSDTECLTRY	KITPRRGPPPT
YRADNEVICT	SYYSKLVPLE	HGEIHTSLIN	GRPSADDPSP	QLLEFTSARY	IRLRLQIRIT
LNADLMTLSH	RDLRDLDPV	TRRYYSIKD	ISVGGMCICY	GHASSCPWDE	EAKQLQCQCE
HNTCGESCDR	CCPGYHQQPW	RPGTISSGNE	CEECNCHNKA	KDCYYDSSVA	KERRSLNTAG
QYSGGGVCVN	CSQNTTGINC	ETCIDQYYRP	HKVSPYDDHP	CRPCNCDPVG	SLSSVCIKDD
RHADLANGKW	PGQCPCRKG	AGDKCDRCQF	GYRGFPNCIP	CDCRTVGS LN	EDPCIEPCLC
KKNVEGKNCD	RCKPGFYNLK	ERNPEGCSEC	FCFVSGVCD	SLTWSISQVT	NMSGWLVTDL
MSTNKIRSQQ	DVLGGHRQIS	INNNAVMOQL	TSTYYWAAPE	AYLGNKLTAF	GGFLKYTVSY
DIPVETVDS	LMSHADIIK	GNGLTISTRA	EGLSLQPYEE	YFNVVRLVPE	NFRDFNTRRE
IDRDQLMTVL	ANVTHLLIRA	NYNSAKMAY	R LDSVSLDIA	SPNAIDLAVA	ADVEHCECPQ
GYTGTSCEAC	LPGYYRVDGI	LFGGICQPCE	CHGHASECDI	HGICSVCTHN	TTGDHCEQCL
PGFYGTSPSRG	TPGDCQPCAC	PLSIDSNNFS	PTCHLTDGEE	VVCDQCAPGY	SGSWCERCAD
GYGNPTVPG	GTCVPCNCSG	NVDPLEAGHC	DSVTGECLKC	LWNTDGAHCE	RCADGFYGD
VTAKNCRACD	CHENGSLSGV	CHLETGLCDC	KPHVTGQQCD	QCLSGYGYLD	TGLGCVPCNC
SVEGVSVDNC	TEEGQCHCGP	GVSGKQCDRC	SHGFYAFQDG	GCTPCDCAHT	QNNCDPASGE
CLCPPHTQGL	KCEECEAYW	GLDPEQGCQA	CNCSAVGSTS	AQCDVLSGHC	PCKKGFGGQS
CHQCSLGYS	FPDCVPCGCD	LRGTLPTDCTD	LEQGLCSCSE	DSGTCSCKEN	VVGPQCSKCQ
AGTFALRGDN	PQGCSPCFCF	GLSQLCSELE	GYVRTLITLA	SDQPLLHVVS	QSNLKG TIEG
VHFQPPDTLL	DAEAVRQHIY	AEPFYWRLPK	QFQGDQLLAY	GKGLQYSVAF	YSTLGTGTSN
YEPQVLIKGG	RARKHVIYMD	APAPENGVRQ	DYEVQMKEEF	WKYFNSVSEK	HVTHSDFMSV
LSNIDYILIK	ASYGQGLQQS	RIANISMEVG	RKAVELPAEG	EAALLLELCV	CPPGTAGHSC
QDCAPGYRE	KLPESGGRGP	RPLLAPCVPC	NCNNHSDVCD	PETGKCLSCR	DHTSGDHCEL
CASGYGKVT	GLPGDCTPCT	CPHHPFFSFS	PTCVVEGDSD	FRCNACLPGY	EGQYCERCSA
GYHGNPRAAG	GSCQTCDCNP	QGSVHSDCDR	ASGQCVCKPG	ATGLHCEKCL	PRHILMESDC
VSCDDDCVGP	LLNDLDSVGD	AVLSLNLTVG	SPAPYGILEN	LENTTKYFQR	YLIKENAKKI
RAEIQLEGIA	EQTENLQKEL	TRVLRHQKV	NAEMERTSNG	TQALATFIEQ	LHANIKEITE
KVATLNQTAR	KDFQPPVSAL	QSMHQNISSL	LGLIKERNFT	EMQONATLEL	KAAKDLLSRI
QKRFPQKPEK	LKALKEANSL	LSNHSEKLQA	AEELLKEAGS	KTOESNLLLL	LVKANLKEEF
QEKKL RVQEE	QNVTS ELIAK	GREWVDAAGT	HTAAAQDTLT	QLEHHRDELL	LWARKIRSHV
DDLVMQMSKR	RARDLVHRAE	QHASELQSR	GALDRDLENV	RNVSLNATSA	AHVHSNIQTL
TEEAEMLAAD	AHKTANKTDL	ISESLASRGK	AVLQRSSRFL	KESVGTRRKQ	QGITMKLDEL
KNLTSQFQES	VDNITKQAND	SLAMLRESPG	GMREKGRKAR	ELAAAANESA	VKTLEDVLAL
SLRVFNTSED	LSRVNATVQE	TNDLLHNSTM	TTLLAGRKMK	DMEQANLLL	DRLKPLKTLE
ENLSRNLSEI	KLLISRARKQ	AASIKVAVSA	DRDCIRAYQP	QTSSTNYNTL	ILNVKTQEPD
NLLFYLGSSS	SSDFLAVEMR	RGKVAFLWDL	SGSSTRLEFP	EVSINNNRWH	SIYITRFGNM
GSLSVKEASA	AENPPVRTSK	SPGPSKVLDI	NNSTLMFVGG	LGGQIKKSPA	VKVTHFKGCM
GEAFLNGKSI	GLWNYIEREG	KCNGCFGSSQ	NEDSSFHFDG	SGYAMVEKTL	RPTVTQIVIL
FSTFSPNGLL	FYLASNGTKD	FLSIELVRGR	VKVMVDLGSG	PLTLMTDRRY	NNGTWYKIAF
QRNRKQGLLA	VFDAYDTSK	ETKQGETPGA	ASDLNRLEKD	LIYVGGLPHS	KAVRKGVS SR
SYVGCICKLE	ISRSTFDLLR	NSYGVKRGCA	LEPIQSVSFL	RGGYVEMPPK	SLSPESSLLA
TFATKNSSGI	LLVALGKDAE	EAGGAQAHVP	FFSIMLLEGR	IEVHVNSGDG	TSLRKALLHA
PTGSYSDGQE	HSISLVRNRR	VITIQVDENS	PVEMKLGPLT	EGKTIDISNL	YIGGLPEDKA
TPMLKMRTSF	HGCIKNVVDL	AQLLDFTHAT	GSEQVELDTC	LLAEPMQSL	HREHGELPPE
PPTLPQPELC	AVDTAPGYVA	GAHQFGLSQN	SHLVLP LNQS	DVRKRLQVQL	SIRTFASSGL
IYYYVAHQNM	DYATLQLQEG	RLHFMFDLGK	GRTKVSHPAL	LSDGKWHTVK	TEYIKRKAFM
TVDGQESPSV	TVVGNATTLD	VERKLYLGGL	PSHYRARNIG	TITHSIPACI	GEIMVNGQQL
DKDRPLSASA	VDRCYVVAQE	GTFEFGSGYA	ALVKEGYKVR	LDLNITLEFR	TTSKNGVLLG
ISSAKVDAIG	LEIVDGKVL F	HVNNGAGRIT	ATYQPRAARA	LCDGKWHTLQ	AHKSNGHRIVL
TVDGNSVRAE	SPHTHST SAD	TNDPIYVGGY	PAHIKQNCLS	SRASFRGCVR	NLRLSRGSQV
QSLDLSRAFD	LQGVFP HSCP	GPEP			

FIGURE 10

SUBSTITUTE SHEET (RULE 28)

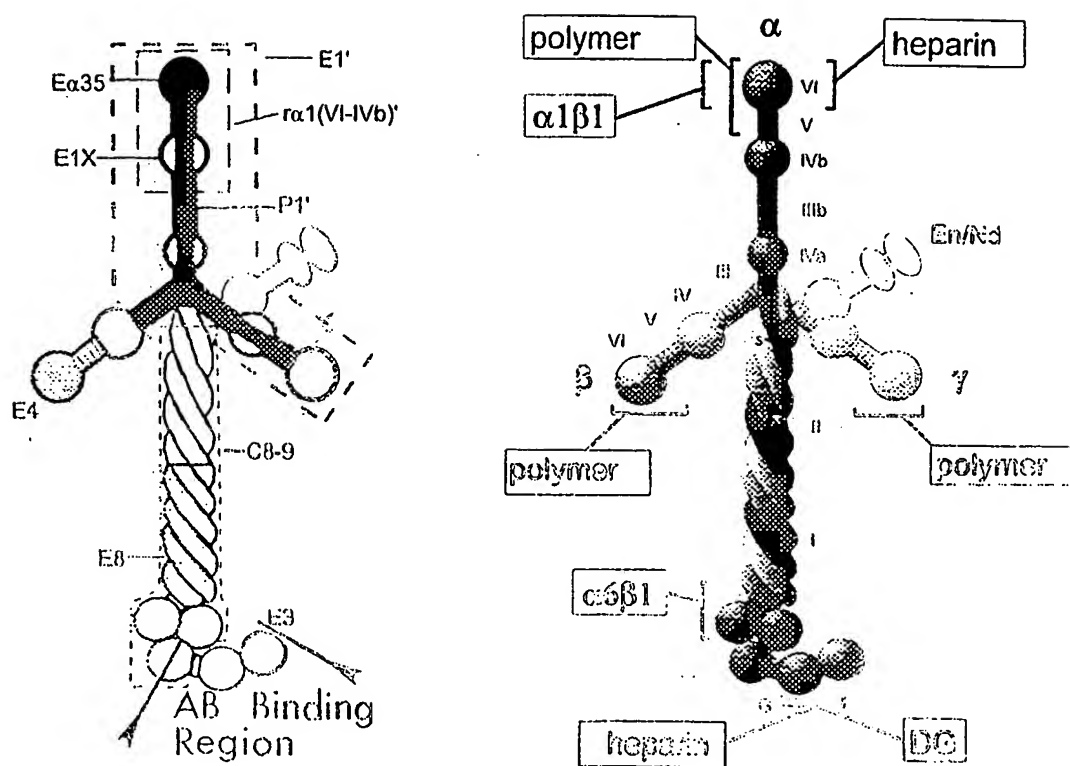


FIGURE 11

SUBSTITUTE SHEET (RULE 26)

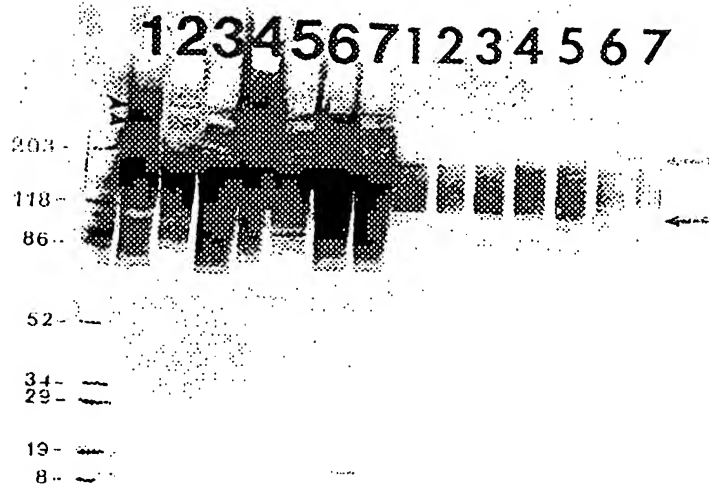


FIGURE 12

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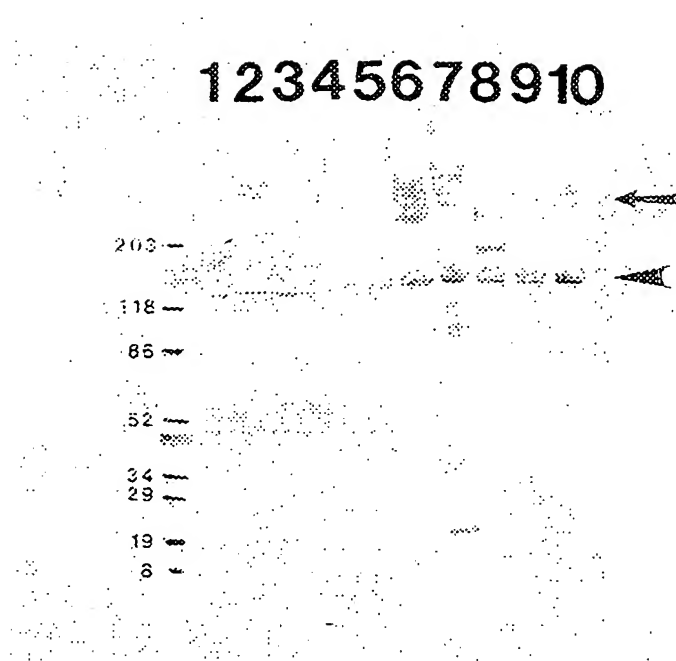


FIGURE 13

SUBSTITUTE SHEET (RULE 20)

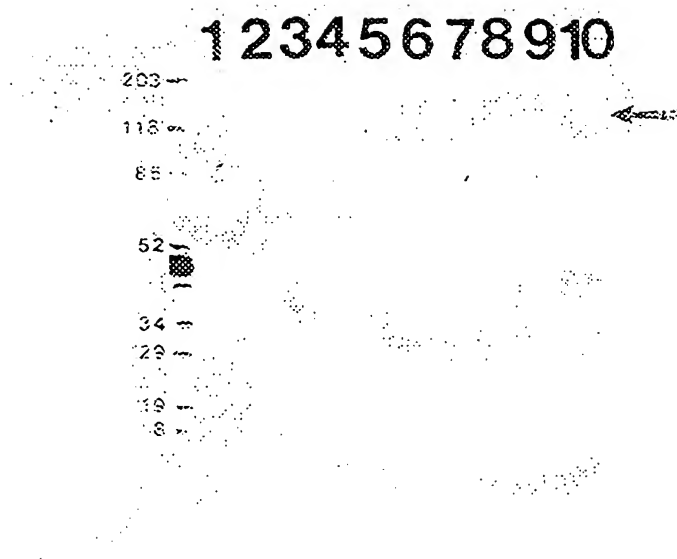


FIGURE 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/18145

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : 514/2, 44; 530/350, 387.1; 435/4, 6, 7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 44; 530/350, 387.1; 435/4, 6, 7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG: author and word. search terms include: laminin, amyloid?, disease. databases: medline, biosis, embase, wpi, uspatful

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KOO et al. Amyloid b-protein as a substrate interacts with extracellular matrix to promote neurite outgrowth. Proc. Natl. Acad. Sci. May 1993, Vol. 90, pages 4748-4752, see entire document.	1-36
Y	NARINDRASORSAK et al. Characterization of High Affinity Binding between Laminin and Alzheimer's Disease Amyloid Precursor Proteins. Laboratory Investigation. 1992, Vol. 67, No. 5, pages 643-652, see entire document.	1-36

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

03 DECEMBER 1997

Date of mailing of the international search report

07 JAN 1998

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Authorized officer

HEATHER BAKALYAR

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/18145

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NARINDRASORASAK et al. An Interaction between Basement Membrane and Alzheimer Amyloid Precursor Proteins Suggests a Role in the Pathogenesis of Alzheimer's Disease. Laboratory Investigation. 1995, Vol. 72, No. 3, pages 272-282, see entire document.	1-36

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/18145

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A01N 37/18, 43/04; A61K 38/00, 31/70; C07K 1/00, 14/00, 17/00, 16/00; C12Q 1/00, 1/68; G01N 33/53